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APPLICATION NUMBER: ***60/602,817***
FILING DATE: ***August 18, 2004***

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Aiping H.	Young	Toronto, Canada

Additional inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Antisense Oligonucleotides Directed to Ribonucleotide Reductase R2 and Uses Thereof in Combination Therapies for the Treatment of Cancer

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 101 ☐ CD(s), Number _____

☑ Drawing(s) *Number of Sheets* 32 ☑ Other (specify) return receipt postcard

☐ Application Data Sheet. See 37 CFR 1.76

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☑ Applicant claims small entity status. See 37 CFR 1.27.

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☑ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE Maria Ellseeva

Date August 18, 2004

REGISTRATION NO. 43,328 *(if appropriate)*

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USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

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ANTISENSE OLIGONUCLEOTIDES DIRECTED TO RIBONUCLEOTIDE REDUCTASE R2 AND USES THEREOF IN COMBINATION THERAPIES FOR THE TREATMENT OF CANCER

FIELD OF THE INVENTION

The present invention pertains to the field of cancer therapeutics and in particular to combinations of an antisense oligonucleotide and one or more immunotherapeutic agents for the treatment of cancer.

BACKGROUND

Regulation of ribonucleotide reductase, and particularly the R2 component, is altered in malignant cells exposed to some tumour promoters and to the growth factor TGF-β [Amara, et al., 1994; Chen et al., 1993; Amara et al., 1995b; Hurta and Wright, 1995; Hurta et al., 1991]. Higher levels of enzyme activity have been observed in cultured malignant cells when compared to nonmalignant cells [Weber, 1983; Takeda and Weber, 1981; Wright et al., 1989a], and increased levels of R2 protein and R2 mRNA have been found in pre-malignant and malignant tissues as compared to normal control tissue samples [Saeki et al., 1995; Jensen et al., 1994]. However, these correlative studies did not show a direct role for ribonucleotide reductase in cancer cell transformation and tumor progression, because like so many other enzyme activities found to be altered in cancer cells [e.g. Weber, 1983], the results could easily be explained by the increased cell proliferation and altered cell cycle regulation characteristics of transformed and malignant cell populations [Morgan and Kastan, 1997].

Antisense oligonucleotides directed to the R1 or R2 component of ribonucleotide reductase have been shown to be effective in reducing the growth of cancer cells [see, for example, U.S. Patent Nos. 5,998,383 and 6,121,000].

In view of the high incidence of various types of cancer throughout the world, there remains a need for improved therapies for the treatment of cancer.

This background information is provided for the purpose of making known information believed by the applicant to be of possible relevance to the present invention. No admission is necessarily intended, nor should be construed, that any of the preceding information constitutes prior art against the present invention.

SUMMARY OF THE INVENTION

An object of the present invention is to provide antisense oligonucleotides directed to ribonucleotide reductase R2 and uses thereof in combination therapies for the treatment of cancer. In accordance with an aspect of the present invention, there is provided a method of treating cancer in a mammal comprising administering to said mammal a combination comprising: (a) one or more antisense oligonucleotides comprising a sequence of at least 7 contiguous nucleotides that are complementary to portion of the mammalian ribonucleotide reductase R2 mRNA, and (b) one or more immunotherapeutic agents.

In accordance with another aspect of the present invention, there is provided a method of treating early stage renal cancer in a human comprising administering to said human a combination comprising: (a) one or more antisense oligonucleotides comprising a sequence as set forth in SEQ ID NO:1, and (b) one or more cytokines.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts effects of SEQ ID NO:1 in combination therapy on HT-29 colon tumour growth in nude mice;

Figure 2 depicts effects of SEQ ID NO:1 in combination therapy on HT-29 colon tumour growth in nude mice;

Figure 3 depicts effects of SEQ ID NO:1 in combination therapy on HT-29 colon tumour growth in nude mice;

Figure 4 depicts effects of SEQ ID NO:1 in combination therapy on HT-29 colon tumour growth in nude mice;

Figure 5 depicts effects of SEQ ID NO:1 in combination therapy on Caki-1 renal tumour growth in SCID mice;

Figure 6 depicts effects of SEQ ID NO:1 in combination therapy on prostatic tumour growth in SCID mice;

Figure 7 depicts effects of SEQ ID NO:1 in combination therapy on prostatic tumour growth in SCID mice;

Figure 8 depicts effects of SEQ ID NO:1 in combination therapy on A2058 melanoma growth in nude mice;

Figure 9 depicts effects of SEQ ID NO:1 in combination therapy on breast tumour growth in CD-1 nude mice;

Figure 10 depicts effects of SEQ ID NO:1 in combination therapy on ovary tumour growth in CD-1 nude mice;

Figure 11 depicts effects of SEQ ID NO: 1 in the treatment of human pancreatic carcinoma in CD-1 nude mice;

Figure 12 depicts effects of SEQ ID NO: 1 in the treatment of human cervix epitheloid carcinoma resistant to hydroxyurea (HU) in SCID mice;

Figure 13 depicts effects of SEQ ID NO: 1 in the treatment of human breast adenocarcinoma resistant to cisplatin in SCID mice;

Figure 14 depicts effects of SEQ ID NO: 1 in the treatment of human breast adenocarcinoma resistant to cisplatin in SCID mice;

Figure 15 depicts effects of SEQ ID NO: 1 in the treatment of human breast adenocarcinoma resistant to taxol in SCID mice;

Figure 16 depicts effects of SEQ ID NO: 1 in the treatment of human breast adenocarcinoma resistant to taxol in SCID mice;

Figure 17 depicts effects of SEQ ID NO: 1 in the treatment of human promyelocytic leukaemia resistant to taxol in SCID mice;

Figure 18 depicts effects of SEQ ID NO: 1 in the treatment of LS513, human multi-drug resistant colon adenocarcinoma in SCID mice;

Figure 19 depicts the anti-proliferative effects of interferon alpha *in vitro* in human renal carcinoma cell lines (Caki-1 and A498);

Figure 20 depicts the effects of SEQ ID NO:1 alone and in combination with interferon alpha on Caki-1 renal tumour growth in SCID mice;

Figure 21 depicts the effects of SEQ ID NO:1 alone and in combination with interferon alpha on Caki-1 renal tumour growth in SCID mice;

Figure 22 depicts the effects of SEQ ID NO:1 alone and in combination with interferon alpha on Caki-1 renal tumour growth in SCID mice;

Figure 23 depicts the effects of SEQ ID NO:1 alone and in combination with interferon alpha on Caki-1 renal carcinoma growth in SCID mice;

Figure 24 depicts the effects of SEQ ID NO:1 alone and in combination with interferon alpha on A498 renal carcinoma growth in SCID mice;

Figure 25 depicts the effects of SEQ ID NO:1 alone and in combination with interferon alpha on A498 renal tumour growth in SCID mice; and

Figure 26 depicts the effects of SEQ ID NO:1 alone and in combination with interleukin-2 on Caki-1 renal tumour growth in SCID mice.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides for combinations comprising one or more antisense oligonucleotides against the gene encoding a mammalian ribonucleotide reductase R2 protein and one or more immunotherapeutic agents for the treatment of cancer. Combinations comprising

antisense oligonucleotides targeted to the ribonucleotide reductase R2 gene and an immunotherapeutic agent have been found to be more effective in decreasing the growth of neoplastic cells than either the antisense oligonucleotide or the immunotherapeutic agent(s) alone. The combinations of the present invention can further comprise one or more chemotherapeutic agents.

In the context of the present invention, a "combination" is used to refer to a combination comprising one or more antisense oligonucleotides and one or more immunotherapeutic agents in multiple, separate dosage units, with each active ingredient of the combination being provided in an individual dosage unit, as well as combinations in multiple dosage units, with each unit comprising one or more active ingredients, and combinations in single dosage units, which contain a fixed ratio of all the active ingredients of the combination. When the combination is provided as more than one dosage unit, individual dosage units can be administered together (concurrently) or separately (sequentially) to the subject undergoing treatment.

The present invention further provides for the use of combinations comprising one or more antisense oligonucleotides targeted to the ribonucleotide reductase R2 gene and one or more immunotherapeutic agent in combination therapies for the treatment of various cancers. The combination therapy can be a first-line treatment against an early stage or newly-diagnosed neoplasm, or it can be a part of an adjuvant therapy for a cancer patient who has already undergone a primary therapy.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains.

The term "antisense oligonculeotide" as used herein refers to an oligonucleotide comprising a sequence that is complementary to the mRNA for a target gene. In the context of the present invention, the target gene is the gene encoding a mammalian ribonucleotide reductase R2 protein.

The term "oligonucleotide," as used herein, means a polymeric form of nucleotides of at least 7 nucleotides in length comprising either ribonucleotides or deoxynucleotides or modified forms of either type of nucleotide. The term includes single and double stranded forms of DNA or RNA.

The term "selectively hybridise" as used herein refers to the ability of a nucleic acid to bind detectably and specifically to a second nucleic acid. Oligonucleotides selectively hybridise to target nucleic acid strands under hybridisation and wash conditions that minimise appreciable amounts of detectable binding to non-specific nucleic acids. High stringency conditions can be used to achieve selective hybridisation conditions as known in the art and discussed herein.

Typically, hybridisation and washing conditions are performed at high stringency according to conventional hybridisation procedures. Washing conditions are typically 1-3 x SSC, 0.1-1% SDS, 50-70°C with a change of wash solution after about 5-30 minutes.

The term "corresponds to" as used herein with reference to nucleic acid sequences means a polynucleotide sequence that is identical to all or a portion of a reference polynucleotide sequence. In contradistinction, the term "complementary to" is used herein to mean that the polynucleotide sequence is identical to all or a portion of the complement of a reference polynucleotide sequence. For illustration, the nucleotide sequence "TATAC" corresponds to a reference sequence "TATAC" and is complementary to a reference sequence "GTATA".

The following terms are used herein to describe the sequence relationships between two or more polynucleotides: "reference sequence," "window of comparison," "sequence identity," "percent (%) sequence identity" and "substantial identity." A "reference sequence" is a defined sequence used as a basis for a sequence comparison; a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA or gene sequence, or may comprise a complete cDNA or gene sequence. Generally, a reference polynucleotide sequence is at least 20 nucleotides in length, and often at least 50 nucleotides in length.

A "window of comparison", as used herein, refers to a conceptual segment of the reference sequence of at least 15 contiguous nucleotide positions over which a candidate sequence may be compared to the reference sequence and wherein the portion of the candidate sequence in the window of comparison may comprise additions or deletions (*i.e.* gaps) of 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The present invention contemplates various lengths for the window of comparison, up to and including the full length of either the reference or candidate sequence. Optimal alignment of sequences for aligning a comparison window may be conducted

using the local homology algorithm of Smith and Waterman (*Adv. Appl. Math.* (1981) 2:482), the homology alignment algorithm of Needleman and Wunsch (*J. Mol. Biol.* (1970) 48:443), the search for similarity method of Pearson and Lipman (*Proc. Natl. Acad. Sci. (U.S.A.)* (1988) 85:2444), using computerised implementations of these algorithms (such as GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 573 Science Dr., Madison, WI), using publicly available computer software such as ALIGN or Megalign (DNASTAR), or by inspection. The best alignment (*i.e.* resulting in the highest percentage of identity over the comparison window) is then selected.

The term "sequence identity" means that two polynucleotide sequences are identical (*i.e.* on a nucleotide-by-nucleotide basis) over the window of comparison.

The term "percent (%) sequence identity," as used herein with respect to a reference sequence is defined as the percentage of nucleotide residues in a candidate sequence that are identical with the residues in the reference polynucleotide sequence over the window of comparison after optimal alignment of the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, without considering any conservative substitutions as part of the sequence identity.

The term "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 50% sequence identity as compared to a reference sequence over the window of comparison. Polynucleotide sequences at least 60% sequence identity, at least 70% sequence identity, at least 80% sequence identity, and at least 90% sequence identity as compared to a reference sequence over the window of comparison are also considered to have substantial identity with the reference sequence.

The terms "therapy," and "treatment," as used interchangeably herein, refer to an intervention performed with the intention of improving a recipient's status. The improvement can be subjective or objective and is related to the amelioration of the symptoms associated with, preventing the development of, or altering the pathology of a disease, disorder or condition being treated. Thus, the terms therapy and treatment are used in the broadest sense, and include the prevention (prophylaxis), moderation, reduction, and curing of a disease, disorder or condition at

various stages. Prevention of deterioration of a recipient's status is also encompassed by the term. Those in need of therapy/treatment include those already having the disease, disorder or condition as well as those prone to, or at risk of developing, the disease, disorder or condition and those in whom the disease, disorder or condition is to be prevented.

The term "ameliorate" or "amelioration" includes the arrest, prevention, decrease, or improvement in one or more the symptoms, signs, and features of the disease being treated, both temporary and long-term.

The term "subject" or "patient" as used herein refers to a mammal in need of treatment.

Administration of the compounds of the invention "in combination with" one or more further therapeutic agents, is intended to include simultaneous (concurrent) administration and consecutive administration. Consecutive administration is intended to encompass administration of the therapeutic agent(s) and the compound(s) of the invention to the subject in various orders.

As used herein, the term "about" refers to a +/-10% variation from the nominal value. It is to be understood that such a variation is always included in any given value provided herein, whether or not it is specifically referred to.

ANTISENSE OLIGONUCLEOTIDES

Selection and Characteristics

The antisense oligonucleotides of the present invention are targeted to the gene encoding a mammalian ribonucleotide reductase R2 protein. The sequences of various mammalian ribonucleotide reductase genes are known in the art, for example, the sequence for the human ribonucleotide reductase R2 gene is provided in Pavloff *et al.* [*J. DNA sequencing and Mapping,* 2;227-234 (1992)]. This and other mammalian R2 sequences are also available from the GenBank database maintained by the NCBI.

The antisense oligonucleotides of the present invention comprise a sequence of at least 7 contiguous nucleotides that are complementary to portion of the mammalian ribonucleotide reductase R2 gene. In one embodiment, the antisense oligonucleotides comprise a sequence of at least 7 contiguous nucleotides that are complementary to portion of the mammalian

ribonucleotide reductase R2 mRNA. In another embodiment, the antisense oligonucleotides comprise a sequence of at least 7 contiguous nucleotides that are complementary to portion of the human ribonucleotide reductase R2 mRNA.

Examples of suitable antisense oligonucleotides for use alone or in the combinations of the present invention include those disclosed in U.S. Patent Nos. 5,998,383 and 6,121,000 (herein incorporated by reference) which are targeted to the ribonucleotide reductase R2 gene. These sequences are provided in Table 1. In one embodiment of the present invention, the antisense oligonucleotides comprise a sequence of at least 7 contiguous nucleotides that are complementary to portion of the coding region of a mammalian ribonucleotide reductase R2 gene or mRNA. In another embodiment, the antisense oligonucleotide comprises at least 7 consecutive nucleotides of any one of the antisense oligonucleotides set forth in Table 1. In a further embodiment, the antisense oligonucleotide comprises at least 7 consecutive nucleotides of the antisense oligonucleotide represented by the sequence:

5'-GGCTAAATCGCTCCACCAAG-3' [SEQ ID NO: 1]

Table 1: Exemplary Antisense Oligonucleotides Targeted to the Human Ribonucleotide Reductase R2 Gene

SEQ ID No	Name	Sequence 5 – 3'	Tm °C	dG kDa/mol
	AS-II-6-20	ACCCTTCCCATTGGCTGCGC	62.8	-45.5
	AS-II-13-20	GsCCsTCCGsACCsCTTCsCCsATTsG	60.1	-43.7
	AS-II-14-20	TGCCTCCGACCCTTCCCATT	60.1	-43.7
	AS-II-16-18	TGCCTCCGACCCTTCCCA	58.4	-40.3
	AS-II-75-20	CsGCGsCGCsTCCsCGGsCCCsTTCsC	72.7	-53.7
	AS-II-75-20	CGCGCGCTCCCGGCCCTTCC	72.7	-53.7
	AS-II-79-14	CGCGCTCCCGGCCC	59.1	-38.8

SEQ ID No	Name	Sequence 5 – 3'	Tm °C	dG kDa/mol
	AS-II-109-20	CsCCCsTCACsTCCsAGCsAGCsCTsT	57.9	-41.8
	AS-II-110-20	ACCCCTCACTCCAGCAGCCT	57.3	-41.2
	AS-II-114-20	GGCGACCCCTCACTCCAGCA	61.8	-43.2
	AS-II-127-12	GCACGGGCGACC	41.7	-28.8
	AS-II-130-20	TGGGACAGGGTGCACGGGCG	67.6	-46.7
	AS-II-134-20	GACGGCTGGGACAGGGTGCA	62.6	-43.2
	AS-II-151-20	GAGCAGCCAGGACAGGACGG	59.3	-41.7
	AS-II-163-20	GsCGsAAGsCAGsAGCsGAGsCAGCsC	62.1	-44.3
	AS-II-166-20	GCAGCGAAGCAGAGCGAGCA	61.4	-43.1
	AS-II-185-20	GGGAGAGCATAGTGGAGGCG	56.0	-40.9
	AS-II-189-20	CGGAGGGAGAGCATAGTGGA	54.1	-39.4
	AS-II-201-20	GCGAGCGGGACACGGAGGGA	63.5	-45.1
	AS-II-217-20	CGGGTCCGTGATGGGCGCGA	69.5	-48.8
	AS-II-225-20	AGCTGCTGCGGGTCCGTGAT	61.4	-43.6
	AS-II-253-14	CCCCTTCAGCGGCG	50.8	-34.4
	AS-II-280-20	CGGCGGCGTGTTCTCCTTGT	61.8	-44.2
	AS-II-288-12	CGGCGGCGTGTT	43.2	-29.6
	AS-II-323-20	TCCTCGCGGTCTTGCTGGCC	64.1	-45.5
	AS-II-344-20	CCGTGGGCTCCTGGAAGATC	58.0	-41.9

SEQ ID No	Name	Sequence 5 – 3'	Tm °C	dG kDa/mol
	AS-II-362-20	CTGCTTTAGTTTTCGGCTCC	51.2	-39.2
	AS-II-391-17	CGGCTCATCCTCCACGC	54.5	-37.3
	AS-II-404-20	GGTTTTCTCTCAGCAGCGGC	56.4	-41.4
	AS-II-412-20	GCGGCGGGGGTTTTCTCTCA	62.8	-45.8
	AS-II-414-20	AAGCGGCGGGGGTTTTCTCT	60.7	-45.8
	AS-II-425-20	GGAAGATGACAAAGCGGCGG	59.1	-43.0
	AS-II-439-20	ATGGTACTCGATGGGGAAGA	50.8	-37.8
	AS-II-472-20	AGCCTCTGCCTTCTTATACA	46.1	-35.8
	AS-II-494-20	CCTCCTCGGCGGTCCAAAAG	60.4	-44.3
	AS-II-496-16	TCCTCGGCGGTCCAAA	54.8	-37.0
	AS-II-549-20	TATCTCTCCTCGGGTTTCAG	48.4	-36.7
	AS-II-579-20	GCAAAGAAAGCCAGAACATG	50.0	-37.2
	AS-II-619-20	TCGCTCCACCAAGTTTTCAT	52.1	-38.3
1	AS-II-626-20	GGCTAAATCGCTCCACCAAG	53.9	-40.3
	AS-II-634-20	AACTTCTTGGCTAAATCGCT	48.0	-37.6
	AS-II-667-20	GAAGCCATAGAAACAGCGGG	53.9	-40.3
	AS-II-784-20	GACACAAGGCATCGTTTCAA	50.9	-36.8
	AS-II-798-20	TCTGCCTTCTTCTTGACACA	48.0	-34.9
	AS-II-816-20	ATCCAGCGCAAGGCCCAGTC	60.9	-43.7

SEQ ID No	Name	Sequence 5 – 3'	Tm °C	dG kDa/mol
	AS-II-861-20	GCAAAGGCTACAACACGTTC	50.0	-37.1
	AS-II-890-20	AACCGGAAAAGAAAATGCCT	52.2	-40.4
	AS-II-909-20	CAGAATATCGACGCAAAAGA	48.2	-36.5
	AS-II-933-20	GGCATCAGTCCTCGTTTCTT	50.8	-37.7
	AS-II-981-20	TGTAAACCCTCATCTCTGCT	46.2	-35.0
	AS-II-1001-20	TCAGGCAAGCAAAATCACAG	51.3	-37.2
	AS-II-1006-20	GAACATCAGGCAAGCAAAAT	49.4	-37.1
	AS-II-1023-20	TTGTGTACCAGGTGTTTGAA	45.9	-33.9
	AS-II-1040-20	CTCTCTCCTCCGATGGTTTG	51.1	-37.7
	AS-II-1048-20	TTCTCTTACTCTCTCCTCCG	45.2	-35.0
	AS-II-1144-20	GTATTGCTTCATTAGAGTGC	41.6	-33.0
	AS-II-1182-20	CCCAGTTCCAGCATAAGTCT	48.4	-36.5
	AS-II-1197-20	AAAACCTTGCTAAAACCCAG	48.3	-37.8
	AS-II-1217-20	CAAATGGGTTCTCTACTCTG	43.7	-33.8
	AS-II-1224-20	ATAAAGTCAAATGGGTTCTC	42.6	-34.0
	AS-II-1254-20	TTAGTCTTTCCTTCCAGTGA	43.8	-33.9
	AS-II-1278-20	TCGCCTACTCTCTTCTCAAA	46.8	-35.6
	AS-II-1288-20	CCTCTGATACTCGCCTACTC	45.6	-35.1
	AS-II-1302-20	GACATCACTCCCATCCTCTG	48.7	-35.3

SEQ ID No	Name	Sequence 5 – 3'	Tm °C	dG kDa/mol
	AS-II-1335-20	GCATCCAAGGTAAAAGAATT	45.6	-36.1
	AS-II-1338-20	TCAGCATCCAAGGTAAAAGA	47.4	-35.9
	AS-II-1342-20	GAAGTCAGCATCCAAGGTAA	46.7	-35.3
	AS-II-1345-20	TTAGAAGTCAGCATCCAAGG	47.0	-35.6
	AS-II-1362-20	GCACATCTTCAGTTCATTTA	42.4	-32.8
	AS-II-1364-20	GGGCACATCTTCAGTTCATT	48.9	-36.2
	AS-II-1381-20	AAAAATCAGCCAAGTAAGGG	48.1	-38.0
	AS-II-1390-20	ATGGAAAAAAAAAATCAGCC	48.1	-38.0
	AS-II-1438-20	TTCATGGTGTGGCTAGTTGG	50.8	-36.8
	AS-II-1499-20	AGGACTGGTTGTGAGGTAGC	48.1	-35.7
	AS-II-1517-20	CCAGCACTATAAACAGACAG	42.2	-32.8
	AS-II-1538-20	TTCTGGCAAAAGGTGATACT	46.5	-35.6
	AS-II-1560-20	GTAAGTCACAGCCAGCCAGG	52.2	-37.8
	AS-II-1581-20	ACTGCCATTGTCACTGCTAT	47.0	-34.9
	AS-II-1659-20	TGGCTGTGCTGGTTAAAGGA	53.2	-38.7
	AS-II-1666-20	TTTTAACTGGCTGTGCTGGT	50.0	-37.2
	AS-II-1700-20	ATTAAAATCTGCGTTGAAGC	46.8	-36.6
	AS-II-1768-20	TATCGCCGCCGTGAGTACAA	56.5	-40.9
	AS-II-1773-20	GCTATTATCGCCGCCGTGAG	57.1	-42.6

SEQ ID No	Name	Sequence 5 – 3'	Tm °C	dG kDa/mol
	AS-II-1775-12	ATCGCCGCCGTG	42.9	-29.5
	AS-II-1790-20	GAAACCAAATAAATCAAGCT	43.4	-34.9
	AS-II-1819-20	TTAGTGGTCAGGAGAATGTA	41.7	-32.5
	AS-II-1976-20	TGGCACCAACTGACTAATAT	44.5	-34.2
	AS-II-1989-20	CCTGTCTTCTATCTGGCACC	48.6	-36.2
	AS-II-2009-20	GCCACAGGATAAAAACACAA	47.7	-35.9
	AS-II-2026-20	CCCAGGACACTACACAAGCC	51.8	-37.5
	AS-II-2044-20	TCAGAGGGGGCAGAGAATCC	55.4	-40.2
	AS-II-2067-20	TCCTTTATCCCACAACACTC	46.3	-35.0
	AS-II-2083-20	CCTTGCCCTGAGAGATTCCT	52.3	-39.0
	AS-II-2083-20	CsCTsTGsCCsCTsGAsGAsGAsTTsCCsT	52.3	-39.0
	AS-II-2128-20	GGCCCAGATCACCCCTAAAT	54.3	-40.9
	AS-II-2151-20	AAACGGCTTCTCACACATAT	46.3	-35.4
	AS-II-2164-20	GAGAAATAAAATGAAACGGC	46.2	-36.6
	AS-II-2182-20	CGTTGAGGAAAATACAGTGA	45.1	-34.3
	AS-II-2229A-20	GCTCCCACATATGAAAACTC	46.1	-35.2
	AS-II-2372-20	CACACAACCTACTTACACCA	42.7	-32.3

Footnotes for Table 1:

Name includes the following: AS = antisense; II = R2; the first number indicates the first nucleotide position in the R2 mRNA sequence; the second number indicates the length of the sequence segment.

Sequences were fully thioated unless partial thioation is indicated (s)
TM°C = melting temperature of oligonucleotide duplex formed.
dG = free energy values of oligonucleotide-complement dimer formation.

The antisense oligonucleotides in accordance with the present invention are selected such that the antisense sequence exhibits the least likelihood of forming duplexes, hair-pins, dimers, or of containing homooligomer / sequence repeats. The oligonucleotide may further contain a GC clamp. One skilled in the art will appreciate that these properties can be determined qualitatively using various computer modelling programs, for example, the program OLIGO® Primer Analysis Software, Version 5.0 (distributed by National Biosciences, Inc., Plymouth, MN).

In order to be effective, conventional antisense oligonucleotides are typically between 7 and 100 nucleotides in length. In one embodiment of the present invention, the antisense oligonucleotides are between about 7 to about 50 nucleotides in length. In other embodiments, the antisense oligonucleotides are between about 7 to about 35 nucleotides in length, between about 12 to about 25 nucleotides in length.

It is understood in the art that an antisense oligonucleotide need not have 100% identity with the complement of its target sequence. The antisense oligonucleotides in accordance with the present invention have a sequence that is at least about 75% identical to the complement of their target sequence. In one embodiment of the present invention, the antisense oligonucleotides have a sequence that is at least about 90% identical to the complement of the target sequence. In another embodiment, they have a sequence that is at least about 95% identical to the complement of target sequence, allowing for gaps or mismatches of several bases. In a further embodiment, they are at least about 98% isentical to the complement of the target sequence. Identity can be determined, for example, by using the BLASTN program of the University of Wisconsin Computer Group (GCG) software or provided on the NCBI website.

In the context of this invention, an oligonucleotide can be an oligomer or polymer of ribonucleic acid (RNA), deoxyribonucleic acid (DNA), or RNA or DNA mimetics. This term, therefore, includes oligonucleotides composed of naturally-occurring nucleobases, sugars and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally-occurring portions, which function similarly. Such modified or substituted oligonucleotides are often

preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target and increased stability in the presence of nucleases. The oligonucleotide can also be a chimeric oligonucleotide, *i.e.* an oligonucleotide that contains two or more chemically distinct regions, each made up of at least one monomer unit.

As is known in the art, a nucleoside is a base-sugar combination and a nucleotide is a nucleoside that further includes a phosphate group covalently linked to the sugar portion of the nucleoside. In forming oligonucleotides, the phosphate groups covalently link adjacent nucleosides to one another to form a linear polymeric compound, with the normal linkage or backbone of RNA and DNA being a 3' to 5' phosphodiester linkage. Specific examples of antisense compounds useful in this invention include oligonucleotides containing modified backbones or non-natural internucleoside linkages. As defined in this specification, oligonucleotides having modified backbones include both those that retain a phosphorus atom in the backbone and those that lack a phosphorus atom in the backbone. For the purposes of the present invention, and as sometimes referenced in the art, modified oligonucleotides that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleotides.

Exemplary antisense oligonucleotides having modified oligonucleotide backbones include, for example, those with one or more modified internucleotide linkages that are phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

In one embodiment of the present invention, the antisense oligonucleotide comprises one or more phosphorothioate internucleotide linkage. In another embodiment, the antisense oligonucleotide comprises phosphorothioate internucleotide linkages that link the four, five or six 3'-terminal nucleotides of the oligonucleotide. In a further embodiment, the antisense oligonucleotide

comprises phosphorothioate internucleotide linkages that link all the nucleotides of the oligonucleotide.

Exemplary modified oligonucleotide backbones that do not include a phosphorus atom are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. Such backbones include morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulphone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulphamate backbones; methyleneimino and methylenehydrazino backbones; sulphonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

The present invention also contemplates oligonucleotide mimetics in which both the sugar and the internucleoside linkage of the nucleotide units are replaced with novel groups. The base units are maintained for hybridisation with an appropriate nucleic acid target compound. An example of such an oligonucleotide mimetic, which has been shown to have excellent hybridisation properties, is a peptide nucleic acid (PNA) [Nielsen *et al.*, *Science*, **254**:1497-1500 (1991)]. In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza-nitrogen atoms of the amide portion of the backbone.

The present invention also contemplates oligonucleotides comprising "locked nucleic acids" (LNAs), which are conformationally restricted oligonucleotide analogues containing a methylene bridge that connects the 2′-O of ribose with the 4′-C (see, Singh *et al., Chem. Commun.,* 1998, 4:455-456). LNA and LNA analogues display very high duplex thermal stabilities with complementary DNA and RNA, stability towards 3′-exonuclease degradation, and good solubility properties. Synthesis of the LNA analogues of adenine, cytosine, guanine, 5-methylcytosine, thymine and uracil, their oligomerization, and nucleic acid recognition properties have been described (see Koshkin *et al., Tetrahedron,* 1998, 54:3607-3630). Studies of mis-matched sequences show that LNA obey the Watson-Crick base pairing rules with generally improved selectivity compared to the corresponding unmodified reference strands.

Antisense oligonucleotides containing LNAs have been described (Wahlestedt *et al., Proc. Natl. Acad. Sci. U. S. A.,* 2000, 97:5633-5638), which were efficacious and non-toxic. In addition, the LNA/DNA copolymers were not degraded readily in blood serum and cell extracts.

LNAs form duplexes with complementary DNA or RNA or with complementary LNA, with high thermal affinities. The universality of LNA-mediated hybridization has been emphasized by the formation of exceedingly stable LNA:LNA duplexes (Koshkin *et al., J. Am. Chem. Soc.,* 1998, 120:13252-13253). LNA:LNA hybridization was shown to be the most thermally stable nucleic acid type duplex system, and the RNA-mimicking character of LNA was established at the duplex level. Introduction of three LNA monomers (T or A) resulted in significantly increased melting points toward DNA complements.

Synthesis of 2'-amino-LNA (Singh et al., J. Org. Chem., 1998, 63, 10035-10039) and 2'-methylamino-LNA has been described and thermal stability of their duplexes with complementary RNA and DNA strands reported. Preparation of phosphorothioate-LNA and 2'-thio-LNA have also been described (Kumar *et al., Bioorg. Med. Chem. Lett.,* 1998, 8:2219-2222).

Modified oligonucleotides may also contain one or more substituted sugar moieties. For example, oligonucleotides may comprise sugars with one of the following substituents at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Examples of such groups are: $O[(CH_2)_n O]_m CH_3$, $O(CH_2)_n OCH_3$, $O(CH_2)_n NH_2$, $O(CH_2)_n CH_3$, $O(CH_2)_n ONH_2$, and $O(CH_2)_n ON[(CH_2)_n CH_3)]_2$, where n and m are from 1 to about 10. Alternatively, the oligonucleotides may comprise one of the following substituents at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, $SO_2 CH_3$, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. Specific examples include 2'-methoxyethoxy (2'-O--CH_2 CH_2 OCH_3, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) [Martin *et al., Helv. Chim. Acta,* 78:486-504(1995)], 2'-

dimethylaminooxyethoxy ($O(CH_2)_2$ $ON(CH_3)_2$ group, also known as 2'-DMAOE), 2'-methoxy (2'-O--CH_3), 2'-aminopropoxy (2'-OCH_2 CH_2 CH_2 NH_2) and 2'-fluoro (2'-F). In one embodiment of the present invention, the antisense oligonucleotide comprises at least one nucleotide comprising a substituted sugar moiety. In another embodiment, the antisense oligonucleotide comprises at least one 2'-O-(2-methoxyethyl) or 2'-MOE modified nucleotide.

Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar.

Oligonucleotides may also include modifications or substitutions to the nucleobase. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808; The Concise Encyclopedia Of Polymer Science And Engineering, (1990) pp 858-859, Kroschwitz, J. I., ed. John Wiley & Sons; Englisch *et al.*, *Angewandte Chemie, Int. Ed.,* **30**:613 (1991); and Sanghvi, Y. S., (1993) *Antisense Research and Applications*, pp 289-302, Crooke, S. T. and Lebleu, B., ed., CRC Press. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid

duplex stability by 0.6-1.2°C [Sanghvi, Y. S., (1993) *Antisense Research and Applications*, pp 276-278, Crooke, S. T. and Lebleu, B., ed., CRC Press, Boca Raton].

Another oligonucleotide modification included in the present invention is the chemical linkage to the oligonucleotide of one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. Such moieties include, but are not limited to, lipid moieties such as a cholesterol moiety [Letsinger *et al.*, *Proc. Natl. Acad. Sci. USA,* **86**:6553-6556 (1989)], cholic acid [Manoharan *et al.*, *Bioorg. Med. Chem. Let.*, **4**:1053-1060 (1994)], a thioether, *e.g.* hexyl-S-tritylthiol [Manoharan *et al.*, *Ann. N.Y. Acad. Sci.*, **660**:306-309 (1992); Manoharan *et al.*, *Bioorg. Med. Chem. Lett.*, **3**:2765-2770 (1993)], a thiocholesterol [Oberhauser *et al.*, *Nucl. Acids Res.*, **20**:533-538 (1992)], an aliphatic chain, *e.g.* dodecandiol or undecyl residues [Saison-Behmoaras *et al.*, *EMBO J.*, **10**:1111-1118 (1991); Kabanov *et al.*, *FEBS Lett.*, **259**:327-330 (1990); Svinarchuk *et al.*, *Biochimie,* **75**:49-54 (1993)], a phospholipid, *e.g.* di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate [Manoharan *et al.*, *Tetrahedron Lett.*, **36**:3651-3654 (1995); Shea *et al.*, *Nucl. Acids Res.*, **18**:3777-3783 (1990)], a polyamine or a polyethylene glycol chain [Manoharan *et al.*, *Nucleosides & Nucleotides,* **14**:969-973 (1995)], or adamantane acetic acid [Manoharan *et al.*, *Tetrahedron Lett.*, **36**:3651-3654 (1995)], a palmityl moiety [Mishra *et al.*, *Biochim. Biophys. Acta,* **1264**:229-237 (1995)], or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety [Crooke *et al.*, *J. Pharmacol. Exp. Ther.*, **277**:923-937 (1996)].

One skilled in the art will recognise that it is not necessary for all positions in a given oligonucleotide to be uniformly modified. The present invention, therefore, contemplates the incorporation of more than one of the aforementioned modifications into a single oligonucleotide or even at a single nucleoside within the oligonucleotide. The present invention further includes antisense compounds that are chimeric compounds. These oligonucleotides typically contain at least one region wherein the oligonucleotide is modified so as to confer upon the oligonucleotide increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the oligonucleotide may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease that cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly

enhancing the efficiency of oligonucleotide inhibition of gene expression. Consequently, comparable results can often be obtained with shorter oligonucleotides when chimeric oligonucleotides are used, compared to phosphorothioate deoxyoligonucleotides hybridising to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridisation techniques known in the art.

In the context of the present invention, an antisense oligonucleotide is "nuclease resistant" when it has either been modified such that it is not susceptible to degradation by DNA and RNA nucleases or alternatively has been placed in a delivery vehicle which in itself protects the oligonucleotide from DNA or RNA nucleases. Nuclease resistant oligonucleotides include, for example, methyl phosphonates, phosphorothioates, phosphorodithioates, phosphotriesters, and morpholino oligomers. Suitable delivery vehicles for conferring nuclease resistance include, for example, liposomes. In one embodiment of the present invention, the antisense oligonucleotides are nuclease resistant.

The present invention further contemplates antisense oligonucleotides that contain groups for improving the pharmacokinetic properties of the oligonucleotide, or groups for improving the pharmacodynamic properties of the oligonucleotide.

Preparation of the Antisense Oligonucleotides

The antisense oligonucleotides of the present invention can be prepared by conventional techniques well-known to those skilled in the art. For example, the oligonucleotides can be prepared using solid-phase synthesis using commercially available equipment, such as the equipment available from Applied Biosystems Canada Inc., Mississauga, Canada. As is well-known in the art, modified oligonucleotides, such as phosphorothioates and alkylated derivatives, can also be readily prepared by similar methods.

Alternatively, the antisense oligonucleotides of the present invention can be prepared by enzymatic digestion of the naturally occurring ribonucleotide reductase R2 gene by methods known in the art.

Antisense oligonucleotides can also be prepared through the use of recombinant methods in which expression vectors comprising nucleic acid sequences that encode the antisense oligonucleotides are expressed in a suitable host cell. Such expression vectors can be readily constructed using procedures known in the art. Examples of suitable vectors include, but are not limited to, plasmids, phagemids, cosmids, bacteriophages, baculoviruses and retroviruses, and DNA viruses. One skilled in the art will understand that selection of the appropriate host cell for expression of the antisense oligonucleotide will be dependent upon the vector chosen. Examples of host cells include, but are not limited to, bacterial, yeast, insect, plant and mammalian cells.

One skilled in the art will also understand that the expression vector may further include regulatory elements, such as transcriptional elements, required for efficient transcription of the antisense oligonucleotide sequences. Examples of regulatory elements that can be incorporated into the vector include, but are not limited to, promoters, enhancers, terminators, and polyadenylation signals. One skilled in the art will appreciate that selection of suitable regulatory elements is dependent on the host cell chosen for expression of the antisense oligonucleotide and that such regulatory elements may be derived from a variety of sources, including bacterial, fungal, viral, mammalian or insect genes.

In accordance with the present invention, the expression vectors can be introduced into a suitable host cell or tissue by one of a variety of methods known in the art. Such methods can be found generally described in Sambrook *et al.*, 1992; Ausubel *et al.*, 1989; Chang *et al.*, 1995; Vega *et al.*, 1995; and Vectors: A Survey of Molecular Cloning Vectors and Their Uses (1988) and include, for example, stable or transient transfection, lipofection, electroporation, and infection with recombinant viral vectors.

IMMUNOTHERAPEUTIC AGENTS

The present invention contemplates the use of one or more immunotherapeutic agents in combination with one or more antisense oligonucleotide against ribonucleotide reductase R2. Immunotherapy is a therapy that directly or indirectly stimulates or enhances the immune system's responses to cancer cells and/or lessens the side effects that may have been caused by other anti-cancer agents. Immunotherapy is also referred to as immunologic therapy, biological

therapy, biological response modifier therapy and biotherapy. The term "immunotherapeutic agent," as used herein, refers to a compound, composition or treatment that indirectly or directly enhances, stimulates or augments the body's immune response against cancer cells and/or that lessens the side effects of other anticancer therapies. Examples of common immunotherapeutic agents known in the art include, but are not limited to, cytokines, cancer vaccines, monoclonal antibodies and non-cytokine adjuvants.

Immunotherapeutic agents can be non-specific, *i.e.* boost the immune system generally so that it becomes more effective in fighting the growth and/or spread of cancer cells, or they can be specific, *i.e.* targeted to the cancer cells themselves. Immunotherapy regimens may combine the use of non-specific and specific immunotherapeutic agents. The present invention contemplates the use of antisense oligonucleotides against ribonucleotide reductase R2 with either non-specific or specific immunotherapeutic agents, or with combinations thereof. In one embodiment, the antisense oligonucleotides are used in combination therapies with one or more non-specific immunotherapeutic agents.

Non-specific immunotherapeutic agents are substances that stimulate or indirectly augment the immune system. Some of these agents can be used alone as the main therapy for the treatment of cancer. Alternatively, non-specific immunotherapeutic agents may be given in addition to a main therapy and thus function as an adjuvant to enhance the effectiveness of other therapies (*e.g.* cancer vaccines) or reduce the side effects of other therapies, for example, bone marrow suppression induced by certain chemotherapeutic agents. Non-specific immunotherapeutic agents can act on key immune system cells and cause secondary responses, such as increased production of cytokines and immunoglobulins. Alternatively, the agents can themselves comprise cytokines. Non-specific immunotherapeutic agents are generally classified as cytokines or non-cytokine adjuvants.

A number of cytokines have found application in the treatment of cancer either as general non-specific immunotherapies designed to boost the immune system, or as adjuvants provided with other therapies. Suitable cytokines for use in the combination therapies of the present invention include interferons, interleukins and colony-stimulating factors.

Interferons (IFNs) contemplated by the present invention for use in combination with the antisense oligonucleotides include the common types of IFNs, IFN-alpha (IFN-α), IFN-beta (IFN-β) and IFN-gamma (IFN-γ). IFNs can act directly on cancer cells, for example, by slowing their growth, promoting their development into cells with more normal behaviour and/or increasing their production of antigens thus making the cancer cells easier for the immune system to recognise and destroy. IFNs can also act indirectly on cancer cells, for example, by slowing down angiogenesis, boosting the immune system and/or stimulating natural killer (NK) cells, T cells and macrophages.

Recombinant IFN-α is available commercially as Róferon (Roche Pharmaceuticals) and Intron A (Schering Corporation). The use of IFN-α, alone or in combination with other immunotherapeutics or with chemotherapeutics, has shown efficacy in the treatment of various cancers including melanoma (including metastatic melanoma), renal cancer (including metastatic renal cancer), breast cancer, prostate cancer, cervical cancer (including metastatic cervical cancer), Kaposi's sarcoma, hairy cell leukemia, chronic myeloid leukemia (CML), multiple myeloma, follicular non-Hodgkin's lymphoma and cutaneous T cell lymphoma.

Interleukins contemplated by the present invention for use in combination with the antisense oligonucleotides include IL-2 (or aldesleukin), IL-4, IL-11 and IL-12 (or oprelvekin). Examples of commercially available recombinant interleukins include Proleukin® (IL-2; Chiron Corporation) and Neumega® (IL-12; Wyeth Pharmaceuticals). Zymogenetics, Inc. (Seattle, WA) is currently testing a recombinant form of IL-21, which is also contemplated for use in the combinations of the present invention. Interleukins, alone or in combination with other immunotherapeutics or with chemotherapeutics, have shown efficacy in the treatment of various cancers including renal cancer (including metastatic renal cancer), melanoma (including metastatic melanoma), ovarian cancer (including recurrent ovarian cancer), cervical cancer (including metastatic cervical cancer), breast cancer, colorectal cancer, lung cancer, brain cancer, prostate cancer, leukemias and lymphomas.

Interleukins have also shown good activity in combination with IFN-α in the treatment of various cancers and the present invention contemplates the use of one or more interleukins and IFN-α in combination therapies with one or more antisense oligonucleotides against ribonucleotide

reductase R2. An interleukin-immunotoxin conjugate known as denileukin diftitox (or Ontak; Seragen, Inc), which comprises IL-2 conjugated to diptheria toxin, has been approved by the FDA for the treatment of cutaneous T cell lymphoma.

Colony-stimulating factors (CSFs) contemplated by the present invention for use in combination with the antisense oligonucleotides include granulocyte colony stimulating factor (G-CSF or filgrastim), granulocyte-macrophage colony stimulating factor (GM-CSF or sargramostim) and erythropoietin (epoetin alfa, darbepoietin). Treatment with one or more growth factors can help to stimulate the generation of new blood cells in patients undergoing traditional chemotherapy. Accordingly, treatment with CSFs can be helpful in decreasing the side effects associated with chemotherapy and can allow for higher doses of chemotherapeutic agents to be used. One embodiment of the present invention provides for the use of higher than standard doses of a chemotherapeutic agent in combination therapies with an antisense oligonucleotide against ribonucleotide reductase R2 and one or more CSFs.

Various recombinant colony stimulating factors are available commercially, for example, Neupogen® (G-CSF; Amgen), Neulasta (pelfilgrastim; Amgen), Leukine (GM-CSF; Berlex), Procrit (erythropoietin; Ortho Biotech), Epogen (erythropoietin; Amgen), Arnesp (erythropoietin). Colony stimulating factors have shown efficacy in the treatment of cancer, including melanoma, colorectal cancer (including metastatic colorectal cancer), lung cancer and leukemia.

Non-cytokine adjuvants suitable for use in the combinations of the present invention include, but are not limited to, levamisole, alum hydroxide (alum), bacillus Calmette-Guerin (BCG), incomplete Freund's Adjuvant (IFA), QS-21, DETOX, Keyhole limpet hemocyanin (KLH) and dinitrophenyl (DNP). Non-cytokine adjuvants in combination with other immuno- and/or chemotherapeutics have demonstrated efficacy against various cancers including, for example, colon cancer and colorectal cancer (Levimasole); melanoma (BCG and QS-21); renal cancer and bladder cancer (BCG).

In addition to having specific or non-specific targets, immunotherapeutic agents can be active, *i.e.* stimulate the body's own immune response, or they can be passive, *i.e.* comprise immune system components that were generated external to the body. Both types of immunotherapeutic

agents are suitable for use with the antisense oligonucleotides against ribonucleotide reductase R2 in the combination therapies of the present invention. In one embodiment, the antisense oligonucleotides are used in combination therapies with one or more active immunotherapeutic agents.

Passive immunotherapy typically involves the use of one or more monoclonal antibodies that are specific for a particular antigen found on the surface of a cancer cell or that are specific for a particular cell growth factor. Monoclonal antibodies may be used in the treatment of cancer in a number of ways, for example, to enhance a subject's immune response to a specific type of cancer, to interfere with the growth of cancer cells by targeting specific cell growth factors, such as those involved in angiogenesis, or by enhancing the delivery of other anticancer agents to cancer cells when linked or conjugated to agents such as chemotherapeutic agents, radioactive particles or toxins.

The present invention contemplates the use of one or more monoclonal antibodies in combination with an antisense oligonucleotide against ribonucleotide reductase R2 for the treatment of cancer. Monoclonal antibodies currently used as cancer immunotherapeutic agents that are suitable for inclusion in the combinations of the present invention include, but are not limited to, rituximab (Rituxan®), trastuzumab (Herceptin®), ibritumomab tiuxetan (Zevalin®), tositumomab (Bexxar®), cetuximab (C-225, Erbitux®), bevacizumab (Avastin®), gemtuzumab ozogamicin (Mylotarg®), alemtuzumab (Campath®), and BL22.

Monoclonal antibodies are used in the treatment of a wide range of cancers including lymphomas (such as non-Hodgkin's lymphoma, B cell chronic lymphocytic leukemia (B-CLL)), myelomas (such as multiple myeloma), leukemias (such as B cell leukemia or acute myelogenous leukemia), breast cancer (including advanced metastatic breast cancer), colorectal cancer (including advanced and/or metastatic colorectal cancer), ovarian cancer, lung cancer, prostate cancer, cervical cancer, melanoma and brain tumors. Monoclonal antibodies can be used alone or in combination with other immunotherapeutic agents or chemotherapeutic agents.

Active specific immunotherapy typically involves the use of cancer vaccines. Cancer vaccines have been developed that comprise whole cancer cells, parts of cancer cells or one or more antigens derived from cancer cells. Cancer vaccines, alone or in combination with one or more

immuno- or chemotherapeutic agents are being investigated in the treatment of several types of cancer including melanoma, renal cancer, ovarian cancer, breast cancer, colorectal cancer, lung cancer and leukemia. Non-specific immunotherapeutics are useful in combination with cancer vaccines in order to enhance the body's immune response. The present invention encompasses combination therapies comprising a cancer vaccine in combination with an antisense oligonucleotide against ribonucleotide reductase R2. The combination may further comprise one or more non-specific immunotherapeutic agents.

CHEMOTHERAPEUTIC AGENTS

As indicated above, the combinations provided by the present invention comprising one or more immunotherapeutic agents and antisense oligonucleotides against ribonucleotide reductase R2 can further comprise one or more chemotherapeutic agents. The chemotherapeutic agent(s) can be selected from a wide range of cancer chemotherapeutic agents known in the art. Known chemotherapeutic agents include those that are specific for the treatment of a particular type of cancer as well as those that are applicable to a range of cancers, such as doxorubicin, capecitabine, mitoxantrone, irinotecan (CPT-11), cisplatin and gemcitabine. Etoposide is generally applicable in the treatment of leukaemias (including acute lymphocytic leukaemia and acute myeloid leukaemia), germ cell tumours, Hodgkin's disease and various sarcomas. Cytarabine (Ara-C) is also applicable in the treatment of various leukaemias, including acute myeloid leukaemia, meningeal leukaemia, acute lymphocytic leukaemia, chronic myeloid leukaemia, erythroleukaemia, as well as non-Hodgkin's lymphoma. Both types of chemotherapeutic agent are suitebl for use in the combinations of the present invention.

Exemplary chemotherapeutics suitable for use in the combinations, which can be used for the treatment specific cancers, are provided in Table 2. One skilled in the art will appreciate that many other chemotherapeutics are available and that the following list is representative only.

TABLE 2: Exemplary Chemotherapeutics used in the Treatment of Some Common Cancers

CANCER	CHEMOTHERAPEUTIC

CANCER	CHEMOTHERAPEUTIC	
Acute lymphocytic leukaemia (ALL)	Pegaspargase (*e.g.* Oncaspar®)	L-asparaginase
	Cytarabine	
Acute myeloid leukaemia (AML)	Cytarabine	Idarubicin
Brain cancer	Procarbazine (*e.g.* Matulane®)	Nitrosoureas
	Platinum analogues	Temozolomide
Breast cancer	Capecitabine (*e.g.* Xeloda®)	Cyclophosphamide
	5-fluorouracil (5-FU)	Carboplatin
	Paclitaxel (*e.g.* Taxol®)	Cisplatin
	Docetaxel (*e.g.* Taxotere®)	Ifosfamide
	Epi-doxorubicin (epirubicin)	Doxorubicin (*e.g.* Adriamycin®)
	Tamoxifen	
Chronic myeloid leukaemia (CML)	Cytarabine	
Colon cancer	Edatrexate (10-ethyl-10-deaza-aminopterin)	
	Methyl-chloroethyl-cyclohexyl-nitrosourea	
	5-fluorouracil (5-FU)	Oxaliplatin
	Fluorodeoxyuridine (FUdR)	Vincristine
	Capecitabine (*e.g.* Xeloda®)	
Colorectal cancer	Irinotecan (CPT-11, *e.g.* Camptosar®)	
	Loperamide (*e.g.* Imodium®)	Levamisole
	Topotecan (*e.g.* Hycamtin®)	Methotrexate
	Capecitabine (*e.g.* Xeloda®)	Oxaliplatin
	5-fluorouracil (5-FU)	
Gall bladder	5-fluorouracil (5-FU)	

CANCER	CHEMOTHERAPEUTIC
Genitourinary cancer	Docetaxel (*e.g.* Taxotere®)
Head and neck cancer	Docetaxel (*e.g.* Taxotere®) Cisplatin
Non-Hodgkin's Lymphoma	Procarbazine (*e.g.* Matulane®) Cytarabine Etoposide
Non-small-cell lung (NSCL) cancer	Vinorelbine Tartrate (*e.g.* Navelbine®) Irinotecan (CPT-11, *e.g.* Camptosar®) Docetaxel (*e.g.* Taxotere®) Paclitaxel (*e.g.* Taxol®) Gemcitabine (*e.g.* Gemzar®) Topotecan
Oesophageal cancer	Porfimer Sodium (*e.g.* Photofrin®) Cisplatin
Ovarian cancer	Irinotecan (CPT-11, *e.g.* Camptosar®) Topotecan (*e.g.* Hycamtin®) Docetaxel (*e.g.* Taxotere®) Paclitaxel (*e.g.* Taxol®) Gemcitabine (*e.g.* Gemzar®) Amifostine (*e.g.* Ethyol®)
Pancreatic cancer	Irinotecan (CPT-11, *e.g.* Camptosar®) Gemcitabine (*e.g.* Gemzar®) 5-fluorouracil (5-FU)
Promyelocytic leukaemia	Tretinoin (*e.g.* Vesanoid®)
Prostate cancer	Goserelin Acetate (*e.g.* Zoladex®) Mitoxantrone (*e.g.* Novantrone®) Prednisone (*e.g.* Deltasone®) Liarozole Nilutamide (*e.g.* Nilandron®) Flutamide (*e.g.* Eulexin®) Finasteride (*e.g.* Proscar®) Terazosin (*e.g.* Hytrin®)

CANCER	CHEMOTHERAPEUTIC	
	Doxazosin (*e.g.* Cardura®)	Cyclophosphamide
	Docetaxel (*e.g.* Taxotere®)	Estramustine
	Luteinizing hormone releasing hormone agonist	
Renal cancer	Capecitabine (*e.g.* Xeloda®)	
	Gemcitabine (*e.g.* Gemzar®)	
Small cell lung cancer	Cyclophosphamide	Vincristine
	Doxorubicin	Etoposide
Solid tumours	Gemicitabine (*e.g.* Gemzar®)	Cyclophosphamide
	Capecitabine (*e.g.* Xeloda®)	Ifosfamide
	Paclitaxel (*e.g.* Taxol®)	Cisplatin
	Docetaxel (*e.g.* Taxotere®)	Carboplatin
	Epi-doxorubicin (epirubicin)	Doxorubicin (*e.g.* Adriamycin®)
	5-fluorouracil (5-FU)	

As indicated above, combinations of chemotherapeutics may be employed. Combination therapies using standard cancer chemotherapeutics are well known in the art and may be included as part of the combinations of the invention. Exemplary combination therapies include for the treatment of breast cancers the combination of epirubicin with paclitaxel or docetaxel, or the combination of doxorubicin or epirubicin with cyclophosphamide. Polychemotherapeutic regimens are also useful and may consist, for example, of doxorubicin/cyclophosphamide/5-fluorouracil or cyclophosphamide/epirubicin/5-fluorouracil. Many of the above combinations are useful in the treatment of a variety of other solid tumours.

Combinations of etoposide with either cisplatin or carboplatin are used in the treatment of small cell lung cancer. In the treatment of stomach or oesophageal cancer, combinations of doxorubicin or epirubicin with cisplatin and 5-fluorouracil are useful. For colorectal cancer, CPT-11 in combination with 5-fluorouracil-based drugs, or oxaliplatin in combination with 5-fluorouracil-based drugs can be used. Oxaliplatin may also be used in combination with capecitabine.

Other examples include the combination of cyclophosphamide, doxorubicin, vincristine and prednisone in the treatment of non-Hodgkin's lymphoma; the combination of doxorubicin, bleomycin, vinblastine and dacarbazine (DTIC) in the treatment of Hodgkin's disease and the combination of cisplatin or carboplatin with any one, or a combination, of gemcitabine, paclitaxel, docetaxel, vinorelbine or etoposide in the treatment of non-small cell lung cancer.

Various sarcomas are treated by combination therapy, for example, for osteosarcoma combinations of doxorubicin and cisplatin or methotrexate with leucovorin are used; for advanced sarcomas etoposide can be used in combination with ifosfamide; for soft tissue sarcoma doxorubicin or dacarbazine can be used alone or, for advanced sarcomas doxorubicin can be used in combination with ifosfamide or dacarbazine, or etoposide in combination with ifosfamide.

Ewing's sarcoma/peripheral neuroectodermal tumour (PNET) or rhabdomyosarcoma can be treated using etoposide and ifosfamide, or a combination of vincristine, doxorubicin and cyclophosphamide.

The alkylating agents cyclophosphamide, cisplatin and melphalan are also often used in combination therapies with other chemotherapeutics in the treatment of various cancers.

EFFICACY OF THE COMBINATIONS OF THE INVENTION

The combinations of antisense oligonucleotides and one or more immunotherapeutic agents can be tested *in vitro* and *in vivo* using standard techniques. Exemplary methods are described below and in the Examples provided herein.

1. In vitro *Testing*

Initial determinations of the ability of the combinations to attenuate the growth or proliferation of neoplastic cells may be made using *in vitro* techniques if required.

For example, the cytotoxicity of the combinations can be assayed *in vitro* using a suitable cancer cell line. In general, cells of the selected test cell line are grown to an appropriate density and the test compound(s) are added. After an appropriate incubation time (for example, about 48 to 72

hours), cell survival is assessed. Methods of determining cell survival are well known in the art and include, but are not limited to, the resazurin reduction test (see Fields & Lancaster (1993) *Am. Biotechnol. Lab.* 11:48–50; O'Brien *et al.*, (2000) *Eur. J. Biochem.* 267:5421-5426 and U.S. Patent No. 5,501,959), the sulforhodamine assay (Rubinstein *et al.*, (1990) *J. Natl. Cancer Inst.* 82:113-118) or the neutral red dye test (Kitano *et al.*, (1991) *Euro. J. Clin. Investg.* 21:53-58; West *et al.*, (1992) *J. Investigative Derm.* 99:95-100). Cytotoxicity is determined by comparison of cell survival in the treated culture with cell survival in one or more control cultures, for example, untreated cultures, cultures pre-treated with a control compound (typically a known therapeutic) and/or cultures treated individually with the components of the combination.

Alternatively, the ability of the combinations to inhibit proliferation of neoplastic cells can be assessed by culturing cells of a cancer cell line of interest in a suitable medium. After an appropriate incubation time, the cells can be treated with the combination and incubated for a further period of time. Cells are then counted and compared to an appropriate control, as described above.

The combinations can also be tested *in vitro* by determining their ability to inhibit anchorage-independent growth of tumour cells. Anchorage-independent growth is known in the art to be a good indicator of tumourigenicity. In general, anchorage-independent growth is assessed by plating cells from an appropriate cancer cell-line onto soft agar and determining the number of colonies formed after an appropriate incubation period. Growth of cells treated with the combinations can then be compared with that of cells treated with an appropriate control (as described above) and with that of untreated cells.

In one embodiment of the present invention, *in vitro* testing of the combinations is conducted in a human cancer cell-line. Examples of suitable cancer cell-lines for *in vitro* testing of the combinations of the present invention include, but are not limited to, non-small cell lung carcinoma cell-lines A549 and H1299, breast cancer cell-line MCF-7, colon cancer cell-lines CaCo, HCT116 and HT29, cervical cancer cell-line HeLa. Other examples of suitable cell-lines are known in the art.

If necessary, the toxicity of the combinations can also be initially assessed *in vitro* using standard techniques. For example, human primary fibroblasts can be treated *in vitro* with the oligonucleotide in the presence of a commercial lipid carrier such as lipofectamine. Cells are then tested at different time points following treatment for their viability using a standard viability assay, such as the trypan-blue exclusion assay. Cells are also assayed for their ability to synthesize DNA, for example, using a thymidine incorporation assay, and for changes in cell cycle dynamics, for example, using a standard cell sorting assay in conjunction with a fluorocytometer cell sorter (FACS).

2. In vivo *Testing*

The ability of the combinations to inhibit tumour growth or proliferation *in vivo* can be determined in an appropriate animal model using standard techniques known in the art (see, for example, Enna, *et al., Current Protocols in Pharmacology,* J. Wiley & Sons, Inc., New York, NY).

In general, current animal models for screening anti-tumour compounds are xenograft models, in which a human or mammalian tumour has been implanted into an animal. Examples of xenograft models of human cancer include, but are not limited to, human solid tumour xenografts in mice, implanted by sub-cutaneous injection and used in tumour growth assays; human solid tumour isografts in mice, implanted by fat pad injection and used in tumour growth assays; experimental models of lymphoma and leukaemia in mice, used in survival assays, and experimental models of lung metastasis in mice.

For example, the combinations can be tested *in vivo* on solid tumours using mice that are subcutaneously grafted bilaterally with a pre-determined amount of a tumour fragment on day 0. The animals bearing tumours are mixed before being subjected to the various treatments and controls. In the case of treatment of advanced tumours, tumours are allowed to develop to the desired size, animals having insufficiently developed tumours being eliminated. The selected animals are distributed at random into groups that will undergo the treatments or act as controls. Suitable groupings would be, for example, those receiving the combination of the invention, those receiving the antisense alone, those receiving the anticancer agent(s) alone and those

receiving no treatment. Animals not bearing tumours may also be subjected to the same treatments as the tumour-bearing animals in order to be able to dissociate the toxic effect from the specific effect on the tumour. Treatment generally begins from 3 to 22 days after grafting, depending on the type of tumour, and the animals are observed every day. The combinations of the present invention can be administered to the animals, for example, by bolus infusion. The different animal groups are weighed about 3 or 4 times a week until the maximum weight loss is attained, after which the groups are weighed at least once a week until the end of the trial.

The tumours are measured about 2 or 3 times a week until the tumour reaches a pre-determined size and / or weight, or until the animal dies if this occurs before the tumour reaches the pre-determined size / weight. The animals are then sacrificed and the tissue histology, size and / or proliferation of the tumour assessed.

For the study of the effect of the compositions on leukaemias, the animals are grafted with a particular number of cells, and the anti-tumour activity is determined by the increase in the survival time of the treated mice relative to the controls.

To study the effect of the combinations of the present invention on tumour metastasis, tumour cells are typically treated with the composition *ex vivo* and then injected into a suitable test animal. The spread of the tumour cells from the site of injection is then monitored over a suitable period of time by standard techniques.

In vivo toxic effects of the oligonucleotides can be evaluated by measuring their effect on animal body weight during treatment and by performing haematological profiles and liver enzyme analysis after the animal has been sacrificed.

Table 3: Examples of xenograft models of human cancer

Cancer Model	Cell Type
Tumour Growth Assay Human solid tumour xenografts in mice (sub-cutaneous injection)	Prostate (PC-3, DU145) Breast (MDA-MB-231, MVB-9) Colon (HT-29) Lung (NCI-H460, NCI-H209) Pancreatic (ASPC-1, SU86.86)

Cancer Model	Cell Type
	Pancreatic: drug resistant (BxPC-3) Skin (A2058, C8161) Cervical (SIHA, HeLa-S3) Cervical: drug resistant (HeLa S3-HU-resistance) Liver (HepG2) Brain (U87-MG) Renal (Caki-1, A498) Ovary (SK-OV-3)
Tumour Growth Assay Human solid tumour isografts in mice (fat pad injection)	Breast: drug resistant (MDA-CDDP-S4, MDA-MB435-To.1)
Survival Assay Experimental model of lymphoma and leukaemia in mice	Human: Burkitts lymphoma (Non-Hodgkin's) (raji) Murine: erythroleukemia (CB7 Friend retrovirus-induced)
Experimental model of lung metastasis in mice	Human: melanoma (C8161) Murine: fibrosarcoma (R3)

In one embodiment of the present invention, the combinations comprising the antisense oligonucleotide with one or more immunotherapeutic agents are more effective than each of the components when used alone. Improved efficacy can be manifested, for example, as a less-than additive effect, wherein the effect of the combination is greater than the effect of each component alone, but less than the sum of the effects of the components, or it may be an additive effect, wherein the effect of the combination is equivalent to the sum of the effects of the components when used individually, or it may be a more-than-additive effect, wherein the effect of the combination is greater than the sum of the effects of each component used alone. Greater than additive effects may also be described as synergistic. The improved efficacy of the combinations can be determined by a number of methods known in the art.

For example, such improved efficacy can result in an increase in the ability of the combination to inhibit the growth or proliferation of neoplastic cells when compared to the effect of each

component alone and/or in lower doses of one or more of the components being required to bring about a certain effect (*i.e.* a decrease in the median effective dose or ED_{50}), and/or decreased toxicity phenomena associated with one or more of the components (*i.e.* a increase in the median lethal dose or LD_{50}). The improved efficacy can also result in an improved therapeutic index or clinical therapeutic index of the combination when compared to the therapeutic index/clinical therapeutic index of each component alone.

As used herein, the term "therapeutic index" is defined as LD_{50} /ED_{50}, where "ED_{50}" is the amount of a compound that produces 50% of the maximum response or effect associated with the compound, or the amount that produces a pre-determined response or effect in 50% of a test population, and "LD_{50}" is the amount of a compound that has a lethal effect in 50% of a test population. Thus, a compound with a high therapeutic index can typically be administered with greater safety than one with a low therapeutic index. The LD_{50} is determined in preclinical trials, whereas the ED_{50} can be determined in preclinical or clinical trials. Preclinical trials are conducted using an appropriate animal model, such as those described herein. The therapeutic index can also be determined based on doses that produce a therapeutic effect and doses that produce a toxic effect (for example, the ED_{90} and LD_{10}, respectively).

"Clinical therapeutic index" differs from the definition of therapeutic index in that some indices of relative safety or relative effectiveness in patients in a clinical setting cannot be defined explicitly and uniquely. A combination is considered to demonstrate an improved Clinical Therapeutic Index, therefore, when it meets one of the following criteria as defined by the Food and Drug Administration: demonstrates increased safety (or patient acceptance) at an accepted level of efficacy within the recommended dosage range, or demonstrates increased efficacy at equivalent levels of safety (or patient acceptance) within the recommended dosage range, as compared to each of the components in the combination. Alternatively, during clinical studies, the dose or the concentration (for example, in solution, blood, serum, plasma) of a drug required to produce toxic effects can be compared to the concentration required to achieve the desired therapeutic effects in the population in order to evaluate the clinical therapeutic index. Methods of clinical studies to evaluate the clinical therapeutic index are well known to workers skilled in the art.

In another embodiment of the present invention, the combinations comprising the antisense oligonucleotide with one or more immunotherapeutic agents exhibit therapeutic synergy, wherein "therapeutic synergy" is demonstrated when a combination is therapeutically superior to one of the components of the combination when used at that component's optimum dose [as defined in T. H. Corbett *et al.*, (1982) *Cancer Treatment Reports*, 66:1187]. To demonstrate the efficacy of a combination, it may be necessary to compare the maximum tolerated dose of the combination with the maximum tolerated dose of each of the separate components in the study in question. This efficacy may be quantified using techniques and equations commonly known to workers skilled in the art [see, for example, T. H. Corbett *et al.*, (1977) *Cancer*, 40, 2660.2680; F. M. Schabel *et al.*, (1979) Cancer Drug Development, Part B, *Methods in Cancer Research,* 17:3-51, New York, Academic Press Inc.].

USE OF THE COMBINATIONS OF THE PRESENT INVENTION

The combinations of the present invention can be used in the treatment of a variety of cancers. In accordance with the present invention, the combination is more effective in reducing the growth and/or metastasis of cancer cells than either the antisense oligonucleotide or the immunotherapeutic agent(s) alone. The present invention contemplates the use of the combinations to treat, stabilize or prevent cancer. In this context, treatment with the combinations may result in a reduction in the size of a tumour, the slowing or prevention of an increase in the size of a tumour, an increase in the disease-free survival time between the disappearance or removal of a tumour and its reappearance, prevention of an initial or subsequent occurrence of a tumour (*e.g.* metastasis), an increase in the time to progression, reduction of one or more adverse symptom associated with a tumour, or an increase in the overall survival time of a subject having cancer.

Examples of cancers which may be may be treated or stabilized in accordance with the present invention include, but are not limited to haematologic neoplasms, including leukaemias and lymphomas; carcinomas, including adenocarcinomas; melanomas and sarcomas. Carcinomas, adenocarcinomas and sarcomas are also frequently referred to as "solid tumours," examples of commonly occurring solid tumours include, but are not limited to, cancer of the brain, breast, cervix, colon, head and neck, kidney, lung, ovary, pancreas, prostate, stomach and uterus, non-

small cell lung cancer and colorectal cancer. Various forms of lymphoma also may result in the formation of a solid tumour and, therefore, are also often considered to be solid tumours.

The term "leukaemia" refers broadly to progressive, malignant diseases of the blood-forming organs. Leukaemia is typically characterized by a distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow but can also refer to malignant diseases of other blood cells such as erythroleukaemia, which affects immature red blood cells. Leukaemia is generally clinically classified on the basis of (1) the duration and character of the disease – acute or chronic; (2) the type of cell involved – myeloid (myelogenous), lymphoid (lymphogenous) or monocytic, and (3) the increase or non-increase in the number of abnormal cells in the blood – leukaemic or aleukaemic (subleukaemic). Leukaemia includes, for example, acute nonlymphocytic leukaemia, chronic lymphocytic leukaemia, acute granulocytic leukaemia, chronic granulocytic leukaemia, acute promyelocytic leukaemia, adult T-cell leukaemia, aleukaemic leukaemia, aleukocythemic leukaemia, basophylic leukaemia, blast cell leukaemia, bovine leukaemia, chronic myelocytic leukaemia, leukaemia cutis, embryonal leukaemia, eosinophilic leukaemia, Gross' leukaemia, hairy-cell leukaemia, hemoblastic leukaemia, hemocytoblastic leukaemia, histiocytic leukaemia, stem cell leukaemia, acute monocytic leukaemia, leukopenic leukaemia, lymphatic leukaemia, lymphoblastic leukaemia, lymphocytic leukaemia, lymphogenous leukaemia, lymphoid leukaemia, lymphosarcoma cell leukaemia, mast cell leukaemia, megakaryocytic leukaemia, micromyeloblastic leukaemia, monocytic leukaemia, myeloblastic leukaemia, myelocytic leukaemia, myeloid granulocytic leukaemia, myelomonocytic leukaemia, Naegeli leukaemia, plasma cell leukaemia, plasmacytic leukaemia, promyelocytic leukaemia, Rieder cell leukaemia, Schilling's leukaemia, stem cell leukaemia, subleukaemic leukaemia, and undifferentiated cell leukaemia.

The term "lymphoma" generally refers to a malignant neoplasm of the lymphatic system, including cancer of the lymphatic system. The two main types of lymphoma are Hodgkin's disease (HD or HL) and non-Hodgkin's lymphoma (NHL). Abnormal cells appear as congregations which enlarge the lymph nodes, form solid tumours in the body, or more rarely, like leukemia, circulate in the blood. Hodgkin's disease lymphomas, include nodular lymphocyte predominance Hodgkin's lymphoma; classical Hodgkin's lymphoma; nodular sclerosis Hodgkin's lymphoma; lymphocyte-rich classical Hodgkin's lymphoma; mixed cellularity

Hodgkin's lymphoma; lymphocyte depletion Hodgkin's lymphoma. Non-Hodgkin's lymphomas include small lymphocytic NHL, follicular NHL; mantle cell NHL; mucosa-associated lymphoid tissue (MALT) NHL; diffuse large cell B-cell NHL; mediastinal large B-cell NHL; precursor T lymphoblastic NHL; cutaneous T-cell NHL; T-cell and natural killer cell NHL; mature (peripheral) T-cell NHL; Burkitt's lymphoma; mycosis fungoides; Sézary Syndrome; precursor B-lymophoblastic lymphoma; B-cell small lymphocytic lymphoma; lymphoplasmacytic lymphoma; spenic marginal zome B-cell lymphoma; nodal marginal zome lymphoma; plasma cell myeloma/plasmacytoma; intravascular large B-cell NHL; primary effusion lymphoma; blastic natural killer cell lymphoma; enteropathy-type T-cell lymphoma; hepatosplenic gamma-delta T-cell lymphoma; subcutaneous panniculitis-like T-cell lymphoma; angioimmunoblastic T-cell lymphoma; and primary systemic anaplastic large T/null cell lymphoma.

The term "sarcoma" generally refers to a tumor which originates in connective tissue, such as muscle, bone, cartilage or fat, and is made up of a substance like embryonic connective tissue and is generally composed of closely packed cells embedded in a fibrillar or homogeneous substance. Sarcomas include soft tissue sarcomas, chondrosarcoma, fibrosarcoma, lymphosarcoma, melanosarcoma, myxosarcoma, osteosarcoma, Abemethy's sarcoma, adipose sarcoma, liposarcoma, alveolar soft part sarcoma, ameloblastic sarcoma, botryoid sarcoma, chloroma sarcoma, chorio carcinoma, embryonal sarcoma, Wilms' tumor sarcoma, endometrial sarcoma, stromal sarcoma, Ewing's sarcoma, fascial sarcoma, fibroblastic sarcoma, giant cell sarcoma, granulocytic sarcoma, Hodgkin's sarcoma, idiopathic multiple pigmented haemorrhagic sarcoma, immunoblastic sarcoma of B cells, lymphoma, immunoblastic sarcoma of T-cells, Jensen's sarcoma, Kaposi's sarcoma, Kupffer cell sarcoma, angiosarcoma, leukosarcoma, malignant mesenchymoma sarcoma, parosteal sarcoma, reticulocytic sarcoma, Rous sarcoma, serocystic sarcoma, synovial sarcoma, and telangiectaltic sarcoma.

The term "melanoma" is taken to mean a tumor arising from the melanocytic system of the skin and other organs. Melanomas include, for example, acral-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, Harding-Passey melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, nodular melanoma, subungal melanoma, and superficial spreading melanoma.

The term "carcinoma" refers to a malignant new growth made up of epithelial cells tending to infiltrate the surrounding tissues and give rise to metastases. Exemplary carcinomas include, for example, acinar carcinoma, acinous carcinoma, adenocystic carcinoma, adenoid cystic carcinoma, carcinoma adenomatosum, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma, basal cell carcinoma, carcinoma basocellulare, basaloid carcinoma, basosquamous cell carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriform carcinoma, cholangiocellular carcinoma, chorionic carcinoma, colorectal carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epiermoid carcinoma, carcinoma epitheliale adenoides, exophytic carcinoma, carcinoma ex ulcere, carcinoma fibrosum, gelatiniform carcinoma, gelatinous carcinoma, giant cell carcinoma, carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, haematoid carcinoma, hepatocellular carcinoma, Hurthle cell carcinoma, hyaline carcinoma, hypemephroid carcinoma, infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, large-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma, melanotic carcinoma, carcinoma molle, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, naspharyngeal carcinoma, oat cell carcinoma, non-small cell carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian carcinoma, scirrhous carcinoma, carcinoma scroti, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tuberous carcinoma, verrucous carcinoma, and carcinoma villosum.

The term "carcinoma" also encompasses adenocarcinomas. Adenocarcinomas are carcinomas that originate in cells that make organs which have glandular (secretory) properties or that

originate in cells that line hollow viscera, such as the gastrointestinal tract or bronchial epithelia. Examples include, but are not limited to, adenocarcinomas of the breast, lung, pancreas and prostate.

Additional cancers encompassed by the present invention include, for example, multiple myeloma, neuroblastoma, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, small-cell lung tumors, primary brain tumors, malignant pancreatic insulanoma, malignant carcinoid, urinary bladder cancer, premalignant skin lesions, gliomas, testicular cancer, thyroid cancer, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, endometrial cancer, adrenal cortical cancer, mesothelioma and medulloblastoma.

As indicated herein, antisense oligonucleotides have been shown to be effective against a wide range of cancers including lymphomas, leukemias and solid tumours, all of which can benefit from treatment with immunotherapeutic agents. One embodiment of the present invention, therefore, provides for the use of the combinations in the treatment of a lymphoma, leukemia or solid tumor. In another embodiment, the present invention provides for the use of the combinations in the treatment of Burkitt's lymphoma, erythroleukemia, acute myeloid leukemia, promyelocytic leukemia, or a solid cancer selected from the group of colon cancer, colorectal cancer, renal cancer, prostate cancer, melanoma, breast cancer, ovarian cancer, pancreatic cancer, cervical cancer and lung cancer. In a further embodiment, the present invention provides for the use of the combinations in the treatment of a cancer that has been shown to respond to immunotherapy, including solid tumours such as melanoma, renal cancer, breast cancer, prostate cancer, cervical cancer, ovarian cancer, colorectal cancer, lung cancer, brain cancer and recurrent and metastatic versions thereof; Kaposi's sarcoma; multiple myeloma; lymphomas such as follicular non-Hodgkin's lymphoma and cutaneous T cell lymphoma; and leukemias such as hairy cell leukemia and chronic myeloid leukemia (CML).

Administration of the Combinations

The combinations of the present invention are administered to a subject in an amount effective to achieve the intended purpose. The exact dosage to be administered can be readily determined by the medical practitioner, in light of factors related to the patient requiring treatment. Dosage and administration are adjusted to provide sufficient levels of each component of the combination

and/or to maintain the desired effect. Factors which may be taken into account when determining an appropriate dosage include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, the particular components of the combination, reaction sensitivities, and tolerance/response to therapy.

In general, determination of the severity of disease requires identification of certain disease characteristics, for example, whether the cancer is pre-metastatic or metastatic, the stage and/or grade of cancer, and the like.

Staging is a process used to describe how advanced a cancer is in a subject. Staging can be important in determining a prognosis, planning treatment and evaluating the results of such treatment. While different cancer staging systems may need to be used for different types of cancer, most staging systems generally involve describing how far the cancer has spread anatomically and attempt to put subjects with similar prognosis and treatment in the same staging group.

Examples of common staging systems used for most solid tumors, some leukemias and lymphomas are the Overall Stage Grouping system and the TMN system. In the Overall Stage Grouping system, Roman numerals I through IV are utilized to denote the four stages of a cancer. Generally, if a cancer is only detectable in the area of the primary lesion without having spread to any lymph nodes it is called Stage I. Stage II and III cancers are generally locally advanced and/or have spread to the local lymph nodes. For example, if the cancer is locally advanced and has spread only to the closest lymph nodes, it is called Stage II. In Stage III, the cancer is locally advanced and has generally spread to the lymph nodes in near proximity to the site of the primary lesion. Cancers that have metastasized from the primary tumor to a distant part of the body, such as the liver, bone, brain or another site, are called Stage IV, the most advanced stage. Accordingly, stage I cancers are generally small localized cancers that are curable, while stage IV cancers usually represent inoperable or metastatic cancers. As with other staging systems, the prognosis for a given stage and treatment often depends on the type of cancer, for example, a stage II non small cell lung cancer has a different prognosis and treatment than a stage II cervical cancer. For some cancers, classification into four prognostic groups is insufficient and the overall staging is further divided into subgroups, for example, with classifications such as IIIa and IIIb. In contrast, some cancers may have fewer than four stage groupings. Additionally, a

cancer that recurs after all visible tumor has been eradicated is called recurrent disease, with local recurrence occurring in the location of the primary tumor and distant recurrence representing distant metastasis. Under this staging system, stage IV can be used interchangeably with distant recurrence.

In the TMN system, the type of tumor, is indicated by T; regional lymph node involvement, is indicated by N; and distant metastases, is indicated by M. Each of the T, N and M categories are classified separately with a number, to determine the total stage. The T category classifies the extent of the primary tumor and is given a number from T0 through to T4, for example, T0 represents a primary tumor that has not invaded the local tissues (*e.g.* in situ), while T4 represents a large primary tumor that may have invaded other organs and is likely inoperable. The N category classifies whether the cancer has metastasized to nearby lymph nodes and is given a number from N0 through to N4, for example, N0 means no lymph node involvement while N4 indicates extensive involvement. The definition of which lymph nodes are regional may depend on the type of cancer. The M category classifies distant metastases and is given a number of M0 or M1, for example, M0 means no distant metastases and M1 indicates distant metastases. As with other staging systems, the exact definition for T and N may differ for each different kind of cancer.

As described above, variations to the staging systems may depend on the type of cancer. For example, most leukemias do not have a staging system as they are not anatomically localized like other solid primary tumors, although a few forms of leukemia do have staging systems to describe the degree of disease advancement. A few leukemias can be defined in stages from I through to IV, but these stages depend, for example, on various factors such as the blood count, extent of bone marrow involvement or the presence or absence of symptoms. Moreover, certain types of cancers, such as prostate cancer or colon cancer, may use staging systems with different nomenclatures, for example, the Duke staging system for colon cancer. The staging system for individual cancers may be revised with new information and subsequently, the resulting stage may change the prognosis and treatment for a specific cancer.

The "grade" of a cancer is used to describe how closely a tumor resembles normal tissue of its same type. Based on the microscopic appearance of a tumor, pathologists identify the grade of a tumor based on parameters such as cell morphology, cellular organization, and other markers of

differentiation. As a general rule, the grade of a tumor corresponds to its rate of growth or aggressiveness and tumors are typically classified from the least aggressive (Grade I) to the most aggressive (Grade IV). Accordingly, the higher the grade, the more aggressive and faster growing the cancer. Information about tumor grade is useful in planning treatment and predicting prognosis.

The American Joint Commission on Cancer has recommended the following guidelines for grading tumors: 1) GX Grade cannot be assessed; 2) G1 Well differentiated; G2 Moderately well differentiated; 3) G3 Poorly differentiated; 4) G4 Undifferentiated. Although grading is used by pathologists to describe most cancers, it plays a more important role in treatment planning for certain types of cancers than for others. An example is the Gleason system that is specific for prostate cancer, which uses grade numbers to describe the degree of differentiation (low scores indicate well differentiated cells and high scores describe poorly differentiated cells). Grade is also important in some types of brain tumors and soft tissue sarcomas.

In accordance with the present invention, the combinations can be used to treat various stages and grades of cancer development and progression. The present invention, therefore, contemplates the use of the combinations in the treatment of early stage cancers including early neoplasias that may be small, slow growing, localized and/or nonaggressive, for example, with the intent of curing the disease or causing regression of the cancer, as well as in the treatment of intermediate stage and in the treatment of late stage cancers including advanced and/or aggressive neoplasias, for example to slow the progression of the disease or to increase the survival of the patient. Similarly, the combinations may be used in the treatment of low grade cancers, intermediate grade cancers and or high grade cancers.

The present invention also contemplates that the combinations can be used in the treatment of indolent cancers, recurrent cancers including locally recurrent, distantly recurrent and/or refractory cancers (*i.e.* cancers that have not responded to treatment), metastatic cancers, locally advanced cancers and aggressive cancers.

One skilled in the art will appreciate that many of these categories may overlap, for example, aggressive cancers are typically also metastatic. "Aggressive cancer," as used herein, refers to a rapidly growing cancer. One skilled in the art will appreciate that for some cancers, such as

breast cancer or prostate cancer the term "aggressive cancer" will refer to an advanced cancer that has relapsed within approximately the earlier two-thirds of the spectrum of relapse times for a given cancer, whereas for other types of cancer, such as small cell lung carcinoma (SCLC) nearly all cases present rapidly growing cancers which are considered to be aggressive. The term can thus cover a subsection of a certain cancer type or it may encompass all of other cancer types.

The combinations may also be used to treat drug resistant cancers, including multidrug resistant tumours. As is known in the art, the resistance of cancer cells to chemotherapy is one of the central problems in the management of cancer.

Certain cancers, such as prostate and breast cancer, can be treated by hormone therapy, *i.e.* with hormonal agents including, for example, hormones or anti-hormone drugs that slow or stop the growth of certain cancers by blocking the body's natural hormones. Such cancers may develop resistance, or be intrinsically resistant, to hormone therapy. The present invention further contemplates the use of the combinations in the treatment of such "hormone-resistant " or "hormone-refractory" cancers.

It is contemplated that the combinations of the present invention can be used alone or in combination with one or more immunotherapeutic agents as part of a primary or adjuvant therapy. "Primary therapy" or "first-line treatment" refers to treatment upon the initial diagnosis of cancer in a subject. Exemplary primary therapies may involve surgery, a wide range of chemotherapies, immunotherapy and radiotherapy. "Adjuvant therapy" refers to a therapy that follows a primary therapy and that is administered to subjects at risk of relapsing. Adjuvant systemic therapy is typically begun soon after primary therapy to delay recurrence, prolong survival or cure a subject. When first-line treatment or primary therapy is not chemotherapeutic or immunotherapeutic, then subsequent adjuvant chemotherapy or immunotherapy may be considered as "first-line chemotherapeutic/ immunotherapeutic treatment." Treatment of refractory cancer may be termed a "second-line treatment" and is a contemplated use of the present invention, in addition to first-line treatment.

In one embodiment of the present invention, the combinations are used in the treatment of an early stage cancer. In another embodiment, the combinations are used as a first-line treatment for an early stage cancer. In an alternate embodiment, the combinations are used in the treatment of a

late stage cancer. In another embodiment, the combinations are used in the treatment of a metastatic cancer.

Antisense oligonucleotides are typically adminstered parenterally, for example, by intravenous infusion. Other methods of administering antisense oligonucleotides are known in the art. Methods of adminstering standard immunotherapeutic agents are also known in the art and include subcutaneous injection, and intravenous, intramuscular or intrasternal injection or infusion techniques.

PHARMACEUTICAL COMPOSITIONS

The antisense oligonucleotide(s) may be administered as a pharmaceutical composition with an appropriate pharmaceutically physiologically acceptable carrier, diluent, excipient or vehicle. The pharmaceutical compositions may also be formulated to contain the antisense oligonucleotide and one or more immunotherapeutic agents for concurrent administration to a patient.

The pharmaceutical compositions of the present invention may be administered orally, topically, parenterally, by inhalation or spray or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques.

The pharmaceutical compositions may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to methods known to the art for the manufacture of pharmaceutical compositions and may contain one or more agents selected from the group of sweetening agents, flavouring agents, colouring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with suitable non-toxic pharmaceutically acceptable excipients including, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, such as corn starch, or alginic acid; binding agents, such as starch, gelatine or acacia, and lubricating agents, such as magnesium stearate, stearic acid or talc. The tablets can

be uncoated, or they may be coated by known techniques in order to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monosterate or glyceryl distearate may be employed.

Pharmaceutical compositions for oral use may also be presented as hard gelatine capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatine capsules wherein the active ingredient is mixed with water or an oil medium such as peanut oil, liquid paraffin or olive oil.

Aqueous suspensions contain the active compound in admixture with suitable excipients including, for example, suspending agents, such as sodium carboxymethylcellulose, methyl cellulose, hydropropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents such as a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example, polyoxyethyene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example, hepta-decaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol for example, polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example, polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or *n*-propyl *p*-hydroxy-benzoate, one or more colouring agents, one or more flavouring agents or one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions may be formulated by suspending the active ingrediients in a vegetable oil, for example, arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example, beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and/or flavouring agents may be added to provide palatable oral preparations. These compositions can be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active compound in admixture with a dispersing or wetting agent,

suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavouring and colouring agents, may also be present.

Pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oil phase may be a vegetable oil, for example, olive oil or arachis oil, or a mineral oil, for example, liquid paraffin, or it may be a mixtures of these oils. Suitable emulsifying agents may be naturally-occurring gums, for example, gum acacia or gum tragacanth; naturally-occurring phosphatides, for example, soy bean, lecithin; or esters or partial esters derived from fatty acids and hexitol, anhydrides, for example, sorbitan monoleate, and condensation products of the said partial esters with ethylene oxide, for example, polyoxyethylene sorbitan monoleate. The emulsions may also contain sweetening and flavouring agents.

Syrups and elixirs may be formulated with sweetening agents, for example, glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, and/or flavouring and colouring agents.

The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to known art using suitable dispersing or wetting agents and suspending agents such as those mentioned above. The sterile injectable preparation may also be sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Acceptable vehicles and solvents that may be employed include, but are not limited to, water, Ringer's solution, lactated Ringer's solution and isotonic sodium chloride solution. Other examples are, sterile, fixed oils which are conventionally employed as a solvent or suspending medium, and a variety of bland fixed oils including, for example, synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Other pharmaceutical compositions and methods of preparing pharmaceutical compositions are known in the art and are described, for example, in "*Remington: The Science and Practice of Pharmacy,*" Gennaro, A., Lippincott, Williams & Wilkins, Philidelphia, PA (2000) (formerly "*Remingtons Pharmaceutical Sciences*").

CLINICAL TRIALS IN CANCER PATIENTS

One skilled in the art will appreciate that, following the demonstrated effectiveness of the combinations of the present invention *in vitro* and in animal models, they should be tested in Clinical Trials in order to further evaluate their efficacy in the treatment of cancer and to obtain regulatory approval for therapeutic use. As is known in the art, clinical trials progress through phases of testing, which are identified as Phases I, II, III, and IV.

Initially the combinations will be evaluated in a Phase I trial. Typically Phase I trials are used to determine the best mode of administration (for example, by pill or by injection), the frequency of administration, and the toxicity for the compounds. Phase I studies frequently include laboratory tests, such as blood tests and biopsies, to evaluate the effects of a compound in the body of the patient. For a Phase I trial, a small group of cancer patients are treated with a specific dose of the antisense oligonucleotide and the one or more chemotherapeutic agent(s). During the trial, the dose is typically increased group by group in order to determine the maximum tolerated dose (MTD) and the dose-limiting toxicities (DLT) associated with the compound. This process determines an appropriate dose to use in a subsequent Phase II trial.

A Phase II trial can be conducted to evaluate further the effectiveness and safety of the combinations. In Phase II trials, the combination is administered to groups of patients with either one specific type of cancer or with related cancers, using the dosage found to be effective in Phase I trials.

Phase III trials focus on determining how a compound compares to the standard, or most widely accepted, treatment. In Phase III trials, patients are randomly assigned to one of two or more "arms". In a trial with two arms, for example, one arm will receive the standard treatment (control group) and the other arm will receive treatment with the combination of the present invention (investigational group).

Phase IV trials are used to further evaluate the long-term safety and effectiveness of a compound. Phase IV trials are less common than Phase I, II and III trials and will take place after the combination has been approved for standard use.

Eligibility of Patients for Clinical Trials

Participant eligibility criteria can range from general (for example, age, sex, type of cancer) to specific (for example, type and number of prior treatments, tumor characteristics, blood cell counts, organ function). Eligibility criteria may also vary with trial phase. For example, in Phase I and II trials, the criteria often exclude patients who may be at risk from the investigational treatment because of abnormal organ function or other factors. In Phase II and III trials additional criteria are often included regarding disease type and stage, and number and type of prior treatments.

Phase I cancer trials usually comprise 15 to 30 participants for whom other treatment options have not been effective. Phase II trials typically comprise up to 100 participants who have already received chemotherapy, surgery, or radiation treatment, but for whom the treatment has not been effective. Participation in Phase II trials is often restricted based on the previous treatment received. Phase III trials usually comprise hundreds to thousands of participants. This large number of participants is necessary in order to determine whether there are true differences between the effectiveness of the combination of the present invention and the standard treatment. Phase III may comprise patients ranging from those newly diagnosed with cancer to those with extensive disease in order to cover the disease continuum.

One skilled in the art will appreciate that clinical trials should be designed to be as inclusive as possible without making the study population too diverse to determine whether the treatment might be as effective on a more narrowly defined population. The more diverse the population included in the trial, the more applicable the results could be to the general population, particularly in Phase III trials. Selection of appropriate participants in each phase of clinical trial is considered to be within the ordinary skills of a worker in the art.

Assessment of patients prior to treatment

Prior to commencement of the study, several measures known in the art can be used to first classify the patients. Patients can first be assessed, for example, using the Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) scale. ECOG PS is a widely accepted standard for the assessment of the progression of a patient's disease as measured by functional

impairment in the patient, with ECOG PS 0 indicating no functional impairment, ECOG PS 1 and 2 indicating that the patients have progressively greater functional impairment but are still ambulatory and ECOG PS 3 and 4 indicating progressive disablement and lack of mobility.

Patients' overall quality of life can be assessed, for example, using the McGill Quality of Life Questionnaire (MQOL) (Cohen *et al* (1995) *Palliative Medicine* 9: 207-219). The MQOL measures physical symptoms; physical, psychological and existential well-being; support; and overall quality of life. To assess symptoms such as nausea, mood, appetite, insomnia, mobility and fatigue the Symptom Distress Scale (SDS) developed by McCorkle and Young ((1978) *Cancer Nursing* 1: 373-378) can be used.

Patients can also be classified according to the type and/or stage of their disease and/or by tumor size.

Administration of the combinations of the present invention in Clinical Trials

The antisense oligonucleotide and the one or more chemotherapeutuc agent(s) are typically administered to the trial participants parenterally. In one embodiment, the combination is administered by intravenous infusion. Methods of administering drugs by intravenous infusion are known in the art. Usually intravenous infusion takes place over a certain time period, for example, over the course of 60 minutes.

Monitoring of Patient Outcome

The endpoint of a clinical trial is a measurable outcome that indicates the effectiveness of a treatment under evaluation. The endpoint is established prior to the commencement of the trial and will vary depending on the type and phase of the clinical trial. Examples of endpoints include, for example, tumour response rate – the proportion of trial participants whose tumour was reduced in size by a specific amount, usually described as a percentage; disease-free survival – the amount of time a participant survives without cancer occurring or recurring, usually measured in months; overall survival – the amount of time a participant lives, typically measured from the beginning of the clinical trial until the time of death. For advanced and/or metastatic cancers, disease stabilisation – the proportion of trial participants whose disease has stabilised,

for example, whose tumour(s) has ceased to grow and/or metastasise, can be used as an endpoint. Other endpoints include toxicity and quality of life.

Tumour response rate is a typical endpoint in Phase II trials. However, even if a treatment reduces the size of a participant's tumour and lengthens the period of disease-free survival, it may not lengthen overall survival. In such a case, side effects and failure to extend overall survival might outweigh the benefit of longer disease-free survival. Alternatively, the participant's improved quality of life during the tumour-free interval might outweigh other factors. Thus, because tumour response rates are often temporary and may not translate into long-term survival benefits for the participant, response rate is a reasonable measure of a treatment's effectiveness in a Phase II trial, whereas participant survival and quality of life are typically used as endpoints in a Phase III trial.

PHARMACEUTICAL KITS

The present invention additionally provides for therapeutic kits containing the antisense oligonucleotide and one or more immunotherapeutic agents in pharmaceutical compositions for use in the treatment of cancer. Individual components of the kit would be packaged in separate containers and, associated with such containers, can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

When the components of the kit are provided in one or more liquid solutions, the liquid solution can be an aqueous solution, for example a sterile aqueous solution. In this case the container means may itself be an inhalant, syringe, pipette, eye dropper, or other such like apparatus, from which the composition may be administered to a patient.

The components of the kit may also be provided in dried or lyophilised form and the kit can additionally contain a suitable solvent for reconstitution of the lyophilised components. Irrespective of the number or type of containers, the kits of the invention also may comprise an instrument for assisting with the administration of the composition to a patient. Such an

instrument may be an inhalant, syringe, pipette, forceps, measured spoon, eye dropper or any such medically approved delivery vehicle.

To gain a better understanding of the invention described herein, the following examples are set forth. It should be understood that these examples are for illustrative purposes only. Therefore, they should not limit the scope of this invention in any way.

EXAMPLES

SEQ ID NO:1 as referred to throughout the Examples is a fully phosphorothioated oligonucleotide with the sequence:
5′-GGCTAAATCGCTCCACCAAG-3′ [SEQ ID NO:1]
SEQ ID NO:1 hybridizes to the coding region of *R2* mRNA.

SEQ ID NO:2 is a mismatched control analogue of SEQ ID NO:1, having four base changes in the middle of the sequence:
5′-GGCTAAACTCGTCCACCAAG-3′ [SEQ ID NO:2]

SEQ ID NO:3 is a scrambled control analogue of SEQ ID NO:1 that is not complementary to R2, but retains the same base composition ratio as SEQ ID NO:1.
5′-ACGCACTCAGCTAGTGACAC-3′ [SEQ ID NO:3]

The phosphorothioates were synthesized on an automated DNA synthesizer (Perkin-Elmer, USA) by Boston BioSystem Inc. (Boston, MA) and were purified by reversed-phase high performance liquid chromatography.

SEQ ID NO:1 is currently being studied in several clinical trials for the treatment of various cancers in combination with standard chemotherapeutic agents as described herein.

EXAMPLE 1: *In vivo* Testing of SEQ ID NO: 1 in Combination with Various Chemotherapeutic Agents in Mouse Xenograft Models

1.1 HT-29 human colon cancer cells ($3X10^6$ cells in 100 µl of PBS) were subcutaneously injected into the right flank of 6-7 weeks old female CD-1 nude mice. After the size of tumour reached an approximate volume of 50 mm^3, 4 days post tumour cell injection, mitomycin C was administered by bolus infusion into the tail vein at days 4, 11 and 18 with a dose of 3.5 mg/kg/week. Antitumour effect of mitomycin C was further compared to that of SEQ ID NO:1 in combination with mitomycin C. SEQ ID NO:1 was administered by bolus infusion into the tail vein every day at 6 mg/kg and mitomycin C was administered intravenously at days 4, 11 and 18 with a dose of 3.5 mg/kg/week, one hour after the treatments with SEQ ID NO:1. Control animals received saline alone for the same period as SEQ ID NO:1. All treatments were stopped at day 22. A day after the last treatment, tumours were excised from the animals and their weights were measured. A standard bar graph was used to demonstrate the differences in tumour weights with each bar representing mean tumour weight calculated from 5 animals. As illustrated, mitomycin C treatments resulted in significant delay of tumour growth compared to saline control. The antitumour effects elicited by the combination of SEQ ID NO:1 and mitomycin C were more potent than those obtained using mitomycin C alone (see Figure 1).

1.2 HT-29 human colon cancer cells ($3X10^6$ cells in 100 µl of PBS) were subcutaneously injected into the right flank of 5-6 week old female CD-1 nude mice. After the size of tumour reached an approximate volume of 100 mm^3, 7 days post tumour cell injection, SEQ ID NO:1 was administered by bolus infusion into the tail vein every other day at 10 mg/kg. Control animals received saline alone for the same period. Antitumour effect of SEQ ID NO:1 was further compared to that of CPT-11 alone or that of SEQ ID NO:1 in combination with CPT-11. CPT-11 was administered intraperitoneally for 5 days in a row from day 7-12 with a dose of 20mg/kg in 100 µl saline. All treatments were stopped at day 32. A day after the last treatment, tumours were excised from the animals and their weights were measured. A standard bar graph was used to demonstrate the differences in tumour weights with each bar representing mean tumour weight calculated from 9 animals. As illustrated, SEQ ID NO:1 treatments resulted in significant delay of tumour growth compared to saline control. The delay in tumour growth achieved with SEQ ID NO:1 was superior to the inhibitory effects observed with CPT-11 alone. The combination treatments of SEQ ID NO:1 and

CPT-11 showed excellent cooperative effects that were more potent than either agent alone (see Figure 2).

1.3 HT-29 human colon cancer cells ($3X10^6$ cells in 100 µl of PBS) were subcutaneously injected into the right flank of 6-7 week old female CD-1 nude mice. Treatments started at day 5 post tumour cell injection, SEQ ID NO:1 and 5-FU were administered as outlined below:

Groups and treatment:

Group 1. Saline treated group: saline:0.1ml/2 days, i. v.;

Group 2. SEQ ID NO:1 treated group: 10 mg/kg/2 days in 100 µL saline, i. v.;

Group 3. 5-FU treated group:13 mg/kg/5 days/week (one week on and one week off), i. v.;

Group 4. SEQ ID NO:1 plus 5-FU treated group.

The growth of human colon tumour cells in CD-1 nude mice treated with SEQ ID NO:1 alone and in combination with 5-FU is depicted in Figure 3.

1.4 HT-29 human colon cancer cells ($3X10^6$ cells in 100 µl of PBS) were subcutaneously injected into the right flank of 5-6 week old female CD-1 nude mice. Treatments started 4 days post tumour cell injection, SEQ ID NO:1 and Capecitabine were administered as outlined below:

Group and Treatment:

Group 1: treated with 0.2 ml vehicle solution for capecitabine, o.p.;

Group 2: treated with 0.1 ml saline, i.v.;

Group 3: treated with 359 mg/kg/day x 5 /w Capecitabine in 0.2 ml vehicle solution, o.p.;

Group 4: treated with 10 mg/kg/2days SEQ ID NO:1 in 0.1 ml saline i.v.;

Group 5: treated with 10 mg/kg/2days SEQ ID NO:1 in 0.1 ml saline, i.v. plus 359 mg/kg/day x 5/w capecitabine in 0.2 ml vehicle solution, o.p. The growth of human colon tumour cells in CD-1 nude mice treated with SEQ ID NO:1 alone and in combination with Capecitabine is depicted in Figure 4.

1.5 Caki-1 human renal cancer cells ($1X10^7$ cells in 100 µl of PBS) were subcutaneously injected into the right flank of 6-7 weeks old female SCID mice. After the size of tumour reached an approximate volume of 200 mm^3, 7 days post tumour cell injection, SEQ ID

NO:1 was administered by bolus infusion into the tail vein every other day at 10 mg/kg. Control animals received saline alone for the same period. The antitumour effect of SEQ ID NO:1 was further compared to that of two chemotherapeutic agents: 5-FU and vinblastin. 5-FU was administered intraperitoneally at days 7-13, 21-27 and 35-36 with a dose of 13 mg/kg/day, while vinblastin was administered intraperitoneally at days 7, 14, 21, 28 and 35 at a dose of 0.6mg/kg/week. Antitumour effects of each of these compounds were further compared to those of SEQ ID NO:1 in combination with 5-FU or with vinblastin. The two chemotherapeutic agents were applied as described above, one hour after the treatments with SEQ ID NO:1 when combination treatments occurred on the same day. All treatments were stopped at day 36. A day after the last treatment, tumours were excised from the animals and their weights were measured. A standard bar graph was used to demonstrate the differences in tumour weights with each bar representing mean tumour weight calculated from 5 animals. As illustrated, SEQ ID NO:1 treatments resulted in significant delay of tumour growth compared to saline control. The delay in tumour growth achieved with SEQ ID NO:1 was superior to the inhibitory effects observed with each of two chemotherapeutic compounds. The combination of SEQ ID NO:1 with 5-FU or vinblastine was more effective than either agent alone (see Figure 5).

1.6 Figure 6 shows results from two independent experiments. In both experiments, PC-3 human prostatic cancer cells ($1X10^7$ cells in 100 µl of PBS) were subcutaneously injected into the right flank of 6-7 weeks old male SCID mice. After the size of tumour reached an approximate volume of 50 mm^3, 14 days post tumour cell injection, SEQ ID NO: 1 was administered by bolus infusion into the tail vein every other day at 10 mg/kg 18 times (Figure 6A) or 17 times (Figure 6B), respectively. Control animals received saline alone for the same period. Antitumour effect of SEQ ID NO:1 was further compared to that of mitoxantrone (novantrone®) alone or in combination. Mitoxantrone was administered intravenously once at the beginning of the treatments at a dose of 2 mg/kg (Figure 6A) or once a week for four weeks at a reduced dose of 0.8 mg/kg (Figure 6B). All treatments were stopped at day 50 (Figure 6A) or 48 (Figure 6B), respectively. A day after the last treatment, tumours were excised from the animals and their weights were measured. A standard bar graph was used to demonstrate the differences in tumour weights with each bar representing mean tumour weight calculated from 5 (Figure 6A) or 10 (Figure 6B) animals. As illustrated in Figure 6A,

SEQ ID NO:1 treatments resulted in significant delay of tumour growth compared to saline control. The delay in tumour growth achieved with SEQ ID NO:1 was similar to the inhibitory effects observed with mitoxantrone alone. The combination of SEQ ID NO:1 with mitoxantrone (SEQ ID NO: 1 +) showed some additive antitumour effects. Figure 6B shows mitoxantrone alone resulted in significant delay of tumour growth and the combination therapy was more potent than mitoxantrone monotherapy.

1.7 Figure 7 shows results from two independent experiments. In both experiments, DU145 human prostatic cancer cells ($1X10^7$ cells in 100 µl of PBS) were subcutaneously injected into the right flank of 6-7 weeks old male SCID mice. After the size of tumour reached an approximate volume of 50 mm^3, 13 (Figure 7A) or 11 (Figure 7B) days post tumour cell injection, SEQ ID NO:1 was administered by bolus infusion into the tail vein every other day at 10 mg/kg 15 times (Figure 7A) or 14 times (Figure 7B), respectively. Control animals received saline alone for the same period. Antitumour effect of SEQ ID NO:1 was further compared to that of mitoxantrone (novantrone®) alone or in combination. Mitoxantrone was administered intravenously once at the beginning of the treatments at a dose of 2 mg/kg (Figure 7A) or once a week for four weeks at a reduced dose of 0.8 mg/kg (Figure 7B). All treatments were stopped at day 42 (Figure 7A) or 38 (Figure 7B), respectively. A day after the last treatment, tumours were excised from the animals and their weights were measured. A standard bar graph was used to demonstrate the differences in tumour weights with each bar representing mean tumour weight calculated from 5 (Figure 7A) or 10 (Figure 7B) animals. As illustrated in Figure 7A, SEQ ID NO:1 treatments resulted in significant delay of tumour growth compared to saline control. The delay in tumour growth achieved with SEQ ID NO:1 was similar to the inhibitory effects observed with mitoxantrone alone. The combination of SEQ ID NO:1 with mitoxantrone (SEQ ID NO: 1 +) showed some additive antitumour effects. In Figure 7B, mitoxantrone alone resulted in significant delay of tumour growth and the combination therapy was more potent than mitoxantrone monotherapy.

1.8 A2058 human melanoma cells ($1X10^7$ cells in 100 µl of PBS) were subcutaneously injected into the right flank of 6-7 week old female CD-1 nude mice. A2058 is a metastatic melanoma cell line. After the size of tumour reached an approximate volume of 100 mm^3, 6 days post tumour cell injection, SEQ ID NO:1 was administered by bolus infusion into the tail vein

every other day at 10 mg/kg. Control animals received saline alone for the same period. Antitumour effect of SEQ ID NO:1 was further compared to that of dacarbazine (DTIC) alone or that of SEQ ID NO:1 in combination with DTIC. DTIC was administered intravenously for 5 days in a row from day 6-10 at a dose of 80mg/kg in 100 µl saline. All treatments were stopped at day 24. A day after the last treatment, tumours were excised from the animals and their weights were measured. A standard bar graph was used to demonstrate the differences in tumour weights with each bar representing mean tumour weight calculated from 10 animals. As illustrated, SEQ ID NO:1 treatments resulted in significant delay of tumour growth compared to saline control. The delay in tumour growth achieved with SEQ ID NO:1 was superior to the inhibitory effects observed with DTIC alone. The combination of SEQ ID NO:1 and DTIC was more potent than either agent alone (Figure 8).

1.9 Figure 9 shows results from three independent experiments. MDA-MB-231 human breast cancer cells ($1X10^7$ cells in 100 µl of PBS) were subcutaneously injected into the right flank of 6-7 weeks old female CD-1 nude mice. After the size of tumour reached an approximate volume of 100 mm^3, 5 days post tumour cell injection, SEQ ID NO:1, or the scrambled control oligonucleotide (SEQ ID NO:3) were administered by bolus infusion into the tail vein every other day at 10 mg/kg. Control animals received saline alone for the same period. Antitumour effect of SEQ ID NO:1 was further compared to that of taxol or doxorubicin alone or in combination. Taxol was administered intravenously once a week at a dose of 10 mg/kg for three (Figure 9A) or four weeks (Figure 9C). Doxorubicin was administered intravenously once a week at a dose of 5 mg/kg for first three weeks (Figure 9A) or for two weeks (Figure 9C). All treatments were stopped at day 33 (Figure 9A) or at day 26 (Figure 9C), respectively. A day after the last treatment, tumours were excised from the animals and their weights were measured. A standard bar graph was used to demonstrate the differences in tumour weights with each bar representing mean tumour weight calculated from 10 animals (Figures 9A & 9B). In Figure 9C, antitumour activities were estimated by the inhibition of tumour volume, which was measured with calipers. Each point represents mean tumour volume calculated from 10 animals per experimental group. As illustrated, SEQ ID NO:1 treatments resulted in a delay of tumour growth compared to saline control in all three experiments. The delay in tumour growth achieved with SEQ ID NO:1 was superior to the inhibitory effects observed with taxol or doxorubicin alone. The combination therapy of SEQ

ID NO: 1 with taxol or doxorubicin was more potent than either monotherapy. Figure 9B demonstrates that a control oligonucleotide that has the same base composition as SEQ ID NO:1, but is not complementary to R2 mRNA has no significant anti-tumour activity as a monotherapy and does not cooperate with doxorubicin, suggesting that the effects of SEQ ID NO:1 are sequence specific.

1.10 SK-OV-3 human ovary adenocarcinoma cells ($1X10^7$ cells in 100 µl of PBS) were subcutaneously injected into the right flank of 6-7 weeks old female CD-1 nude mice. After the size of tumour reached an approximate volume of 100 mm^3, 6 days post tumour cell injection, SEQ ID NO: 1 was administered by bolus infusion into the tail vein every other day at 10 mg/kg 17 times. Control animals received saline alone for the same period. Antitumour effect of SEQ ID NO: 1 was further compared to that of taxol or cisplatin alone or in combination. Taxol was administered intravenously once a week for first three weeks and intraperitoneally once a week for next two weeks at a dose of 10 mg/kg. Cisplatin was administered intravenously once a week for first three weeks and intraperitoneally once a week for next two weeks at a dose of 4 mg/kg. All treatments were stopped at day 40. Antitumour activities were estimated by the inhibition of tumour volume, which was measured with caliper. Each point represents mean tumour volume calculated from 9 animals per experimental group. As illustrated, SEQ ID NO:1 treatments resulted in significant delay of tumour growth compared to saline control. The delay in tumour growth achieved with SEQ ID NO:1 was similar or superior to the inhibitory effects observed with taxol or cisplatin alone, respectively. The combination therapy of SEQ ID NO:1 with taxol or cisplatin was more potent than either monotherapy (Figure 10).

Results of SEQ ID NO:1 treatment in combination with various chemotherapeutic agents are summarized in Table 4.

TABLE 4: Summary of SEQ ID NO:1 Treatment in Combination with Standard Chemotherapy Drugs

Tumour	Mouse	Treatment	Tumour weight as % of saline control
Caki (renal)	CD-1	SEQ ID NO:1	3.3

Tumour	Mouse	Treatment	Tumour weight as % of saline control
		5-FU	52
		Vinblastine	26
		SEQ ID NO:1 + 5-FU	0
		SEQ ID NO: 1 + Vinblastine	0
HT-29 (colon)	SCID	Mitomycin C	15
		SEQ ID NO:1 + Mitomycin C	0.8
HT-29 (colon)	CD-1	SEQ ID NO:1	19
		CPT-11	36
		SEQ ID NO:1 + CPT-11	1.4
MDA-MB-231 (breast)	CD-1	SEQ ID NO:1	12.6
		Taxol	58
		Doxorubicin	41
		SEQ ID NO:1 + Taxol	1
		SEQ ID NO:1 + Doxorubicin	4.8
A2058 (melanoma)	CD-1	SEQ ID NO:1	20
		DTIC	68
		SEQ ID NO:1 + DTIC	8
PC-3 (prostatic)	SCID	Novantrone	57
		SEQ ID NO:1 + Novantrone	21
DU145 (prostatic)	SCID	SEQ ID NO:1	n.a. 41
		Novantrone	40 60
		SEQ ID NO:1 + Novantrone	4.6 23

Tumour	Mouse	Treatment	Tumour weight as % of saline control
**SK-OV-3 (ovary)	CD-1	SEQ ID NO:1	42
		Taxol	49
		Cisplatin	67
		SEQ ID NO:1 + Taxol	24
		SEQ ID NO:1 + Cisplatin	27

Results shown are mean tumour weights presented as a percentage of saline treated controls.

** is tumour volume data as percentage of saline control.

EXAMPLE 2: *In vivo* Testing of SEQ ID NO:1 Alone or in Combination with Various Chemotherapeutic Agents in Drug-Resistant Tumors

2.1 BxPC-3 human pancreatic carcinoma cells ($3X10^6$ cells in 100 µl of PBS) were subcutaneously injected into the right flank of 6-7 weeks old female CD-1 nude mice. BxPC-3 is a gemcitabine resistant call line. After the size of tumour reached an approximate volume of 100 mm^3, 21 days post tumour cell injection, SEQ ID NO: 1 was administered by bolus infusion into the tail vein every other day at 10 mg/kg 17 times. Control animals received saline alone for the same period. Antitumour effect of SEQ ID NO: 1 was further compared to that of Gemcitabine. Gemcitabine was administered intravenously every three days at a dose of 100 mg/kg. Antitumour activities were estimated by the inhibition of tumour volume, which was measured with caliper. Each point represents mean tumour volume calculated from 10 animals per experimental group. As illustrated, SEQ ID NO: 1 treatments resulted in significant delay of tumour growth compared to saline control. As expected, treatment with Gemcitabine during the same period was ineffective against Gemcitabine-resistant tumour (Figure 11A & B).

2.1 Hela S3 human cervix epitheloid carcinoma cells ($5X10^5$ cells in 100 µl of PBS) were subcutaneously injected into the right flank of 6-7 weeks old female SCID mice. Hela S3 is a hydroxyurea resistant cell line. After the size of tumour reached an approximate volume of

100 mm^3, 3 days post tumour cell injection, SEQ ID NO:1 was administered by bolus infusion into the tail vein every other day at 10 mg/kg 6 times. Control animals received saline alone for the same period. Antitumour effect of SEQ ID NO:1 was further compared to that of Hydroxyurea or Cisplatin alone or in combination. Hydroxyurea was administered intraperitoneally every day at a dose of 250 mg/kg for 10 days. Cisplatin was administered intravenously once a week for three weeks at a dose of 4 mg/kg. Antitumour activities were estimated by the inhibition of tumour volume, which was measured with caliper. Each point represents mean tumour volume calculated from 10 animals per experimental group. As illustrated, SEQ ID NO:1 treatments resulted in significant delay of tumour growth compared to saline control. As expected, treatment with Hydroxyurea during the same period was ineffective against Hydroxyurea-resistant tumour. The delay in tumour growth achieved with SEQ ID NO:1 was superior to the inhibitory effects observed with Cisplatin alone, which was used as a positive control. The combination therapy of SEQ ID NO:1 with Hydroxyurea was only as effective as SEQ ID NO: 1 monotherapy, as expected. The combination therapy of SEQ ID NO:1 with Cisplatin, however, was more potent than either monotherapy (Figure 12A & B).

2.3 MDA-CDDP-S4 human *in vivo*-selected Cisplatin-resistant breast adenocarcinoma cells ($4X10^6$ cells in 100 µl of PBS) were injected into the fat pad (inside of right leg) of 6-7 weeks old female SCID mice. After the size of tumour reached an approximate volume of 100 mm^3, 7 days post tumour cell injection, SEQ ID NO: 1 was administered by bolus infusion into the tail vein every other day at 10 mg/kg 9 times. Control animals received saline alone for the same period. Antitumour effect of SEQ ID NO: 1 was further compared to that of Cisplatin or Taxol alone. Cisplatin was administered intravenously once a week for three weeks at a dose of 4 mg/kg. Taxol was administered intravenously once a week for three weeks at a dose of 10 mg/kg. Antitumour activities were estimated by the inhibition of tumour volume, which was measured with caliper. Each point represents mean tumour volume calculated from 10 animals per experimental group. As illustrated, SEQ ID NO: 1 treatments caused significant reduction of tumour weight compared to saline control. As expected, treatment with Cisplatin during the same period was ineffective against Cisplatin-resistant tumour. The delay in tumour growth achieved with SEQ ID NO: 1 was similar to the inhibitory effects observed with Taxol, which was used as a positive control (Figure 13).

2.4 MDA-CDDP-S4 human *in vivo*-selected Cisplatin-resistant breast adenocarcinoma cells ($4X10^6$ cells in 100 µl of PBS) were injected into the fat pad (inside of right leg) of 6-7 weeks old female CB-17 SCID mice. After the size of tumour reached an approximate volume of 100 mm^3, 9 days post tumour cell injection, SEQ ID NO:1 was administered by bolus infusion into the tail vein every other day at 10 mg/kg. Control animals received saline alone for the same period. Antitumour effect of SEQ ID NO: 1 was further compared to that of Taxol alone and in combination. Taxol was administered i.p. once a week at a dose of 10 mg/kg. Antitumour activities were estimated by the inhibition of tumour volume (Figure 14A), which was measured with calipers. Each point represents mean tumour volume calculated from 10 animals per experimental group. Animals were sacrificed and tumour weights taken at the end of the study (Figure 14C). SEQ ID NO:1 treatments caused significant reduction of tumour weight compared to saline control. The delay in tumour growth achieved with SEQ ID NO:1 was superior to the inhibitory effects observed with Taxol, which was used as a positive control. The effects of combined treatment were greater than either treatment alone. This study was repeated with similar results (Figure 14B).

2.5 MDA-MB435-To.1 human Taxol-resistant breast adenocarcinoma cells ($4X10^6$ cells in 100 µl of PBS) were injected into the fat pad (inside of right leg) of 6-7 weeks old female SCID mice. After the size of tumour reached an approximate volume of 100 mm^3, 20 days post tumour cell injection, SEQ ID NO: 1 was administered by bolus infusion into the tail vein every other day at 10 mg/kg 15 times. Control animals received saline alone for the same period. Antitumour effect of SEQ ID NO: 1 was further compared to that of Cisplatin or Taxol alone. Cisplatin was administered intravenously once a week for four weeks at a dose of 4 mg/kg. Taxol was administered intravenously once a week for four weeks at a dose of 20 mg/kg. Antitumour activities were estimated by the inhibition of tumour volume, which was measured with caliper. Each point represents mean tumour volume calculated from 9-10 animals per experimental group. As illustrated, SEQ ID NO:1 treatments caused significant reduction of tumour weight compared to saline control. As expected, treatment with Taxol during the same period was ineffective against Taxol-resistant tumour. The delay in tumour growth achieved with SEQ ID NO:1 was superior to the inhibitory effects observed with Cisplatin, which was used as a positive control (see Figure 15A & B).

2.6 MDA-MB435-To.1 human Taxol-resistant breast adenocarcinoma cells ($4X10^6$ cells in 100 µl of PBS) were injected into the fat pad (inside of right leg) of 6-7 weeks old female CB-17 SCID mice. After the size of tumour reached an approximate volume of 100 mm^3, 17 days post tumour cell injection, SEQ ID NO:1 was administered by bolus infusion into the tail vein every other day at 10 mg/kg. Control animals received saline alone for the same period. Antitumour effect of SEQ ID NO: 1 was compared to that of Cisplatin alone and in combination. Cisplatin was administered intravenously once a week for four weeks at a dose of 4 mg/kg. Antitumour activities were estimated by the inhibition of tumour volume, which was measured with caliper. Each point represents mean tumour volume calculated from 10 animals per experimental group. At the end of the study the animals were sacrificed and tumours weighed. As illustrated, SEQ ID NO:1 treatment caused significant reduction of tumour weight compared to saline control. The delay in tumour growth achieved with SEQ ID NO:1 was superior to the inhibitory effects observed with Cisplatin, which was used as a positive control. The combination of the two compounds produced anti-tumour efficacy that was superior to either one alone (see Figure 16A & B).

2.7 Human taxol-resistant promyelocytic leukaemia cells (HL-60) ($7X10^6$ cells in 100 µl of PBS) were injected into the right flank of 6-7 weeks old female SCID mice. After the size of tumour reached an approximate volume of 100 mm^3, 10 days post tumour cell injection, SEQ ID NO:1 was administered by bolus infusion into the tail vein every other day at 10 mg/kg. Control animals received saline alone for the same period. The anti-tumour effect of SEQ ID NO:1 was further compared to that of taxol. Taxol was administered i.p. once a week at a dose of 10 mg/kg. Anti-tumour activity was estimated by the inhibition of tumour volume, which was measured with caliper. Each point represents mean tumour volume calculated from 10 animals per experimental group. In addition animals were sacrificed and tumour weights taken at the end of the study. SEQ ID NO:1 treatments caused significant reduction of tumour weight compared to saline control. As expected, treatment with taxol had no effect on tumour growth or weight (see Figure 17A & B).

2.8 LS513 multi-drug resistant colon carcinoma cells ($1X10^7$ cells in 100 µl of PBS) were subcutaneously injected into the right flank of 6-7 weeks old female SCID mice. After the size of tumour reached an approximate volume of 100 mm^3, 8 days post tumour cell

injection, SEQ ID NO:1 was administered by bolus infusion into the tail vein every other day at 10 mg/kg. Control animals received saline alone for the same period. Antitumour effect of SEQ ID NO:1 was further compared to that of CPT-11 alone or in combination. CPT-11 was administered i.p. for 5 days at a dose of 20 mg/kg/day. Antitumour activities were estimated by the inhibition of tumour volume, which was measured with caliper. Each point represents mean tumour volume calculated from 10 animals per experimental group. Tumour weights were measured after animals were sacrificed at the end of the treatment. These cells are not resistant to CPT-11 which was used as a positive control. As illustrated, SEQ ID NO:1 treatment resulted in significant delay of tumour growth compared to saline control. SEQ ID NO:1 is as effective as CPT-11 and in combination the efficacy was greater than either treatment alone (see Figure 18A, B & C).

Results of SEQ ID NO:1 treatment of drug-resistant tumours alone or in combination with various chemotherapeutics are summarized in Table 5.

TABLE 5: Summary of SEQ ID NO:1 Treatment of Drug Resistant Tumours

Tumour Resistance	**Mouse**	**Treatment**	**Tumour weight as % of saline control**
LS513 (colon)	SCID	CPT-11	47
multi-drug resistant		SEQ ID NO:1	49
(CPT-11 sensitive)		SEQ ID NO:1 + CPT-11	3
MDA-CDDP-S4	SCID	SEQ ID NO:1	32
(breast)		Taxol	32
Cisplatin		Cisplatin	78
MDA-CDDP-S4	SCID	SEQ ID NO:1	18 41
(breast)		Taxol	26 61
Cisplatin		SEQ ID NO:1 + Taxol	1 12

Tumour Resistance	Mouse	Treatment	Tumour weight as % of saline control
MDA-MB435-To.1 (breast) *Taxol*	SCID	SEQ ID NO:1	42
		Taxol	109
		Cisplatin	69
MDA-MB435-To.1 (breast) *Taxol*	CB-17/ SCID	SEQ ID NO:1	37
		Cisplatin	56
		SEQ ID NO:1 + cisplatin	22
HL-60 (leukemia) *Taxol*	SCID	SEQ ID NO:1	38
		Taxol	119
BxPC-3 (pancreatic) *Gemcitabine*	CD-1	SEQ ID NO:1	5.8
		Gemcitabine	83
Hela S3 (cervix) *hydroxyurea*	SCID	SEQ ID NO:1	24
		Hydroxyurea (HU)	92
		Cisplatin	63
		SEQ ID NO:1 + HU	36
		SEQ ID NO:1+ Cisplatin	13

Results shown are mean tumour weights presented as a percentage of saline treated controls.

EXAMPLE 3: *In vitro* Testing of Interferon Alpha (IFN alpha) Alone in Human Renal Carcinoma Cell Lines

Preliminary *in vitro* testing was performed on human renal carcinoma cell lines (A498 and Caki-1) to determine whether these cell lines were sensitive to the direct anti-proliferative effects of IFN alpha. Cultured cells were treated for 96 hours with increasing concentrations of IFN alpha (0, 100, 600, 800, 1000, 3000, or 10000 U/ml) and cell proliferation was assessed by XTT assay.

The *in vitro* anti-proliferative effects of IFN alpha are shown in Figure 19A (A498) and Figure 19B (Caki-1), which illustrates that both cell lines were sensitive to IFN alpha in a dose-dependent manner.

EXAMPLE 4: *In vivo* Testing of SEQ ID NO: 1 Alone and in Combination with Interferon Alpha in a Mouse Xenograft Model of Human Renal Carcinoma (Caki-1)

Cell Line: Human renal carcinoma cell line (Caki-1) was grown as monolayer culture in Minimum essential medium (α-MEM) supplemented with 10% fetal bovine serum (FBS), 0.1 mM non-essential amino acid, 1.0 mM sodium pyruvate, 100 U/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B and 2mM L-alanyl-l-glutamine at 37°C in an atmosphere of 5% CO_2 in air. The tumor cells were routinely subcultured twice weekly by trypsin-EDTA treatment. The cells were harvested from subconfluent logarithmically growing culture by treatment with trypsin-EDTA and counted for tumor inoculation.

Tumor Inoculation: An acclimation period of at least 7 days was allowed between animal receipt and commencement of tumor inoculation. When the female SCID mice were 6-7 weeks of age (20-25 g), each mouse was subcutaneously (*s.c.*) injected with 5 x 10^6 Caki-1 human renal carcinoma cells in 0.1 ml of PBS at the right flank of the mice to induce tumor growth.

Dose Preparation and Treatment: The dose for SEQ ID NO:1 was formulated using the following procedure. From a concentrated stock solution (usually prepared in ddH_2O at a concentration of 50 mg/ml and kept at -80°C), SEQ ID NO:1 was diluted with saline to achieve a final target concentration of 1 or 2.5 mg/ml on the first day of dosing. Enough SEQ ID NO:1 was diluted so that the compound could be administered by bolus infusion into the tail vein every other day for the duration of the experiment. A standard immunotherapeutic agent for renal carcinoma Intron A™ (recombinant interferon alfa-2b) was utilized. The dose and treatment schedules for Intron A are described below. The treatments were started 7 days after the tumor cell inoculation when the tumor size reached an approximate volume of 125 mm^3. Each treatment group contained 10 tumor-bearing mice. Routes of administration are indicated as follows: intravenous (i.v.) and intratumoral (i.t.).

Groups and Treatments: The following treatment groups were evaluated:

1. Saline treated group-1: saline 0.1ml/mouse/48 hours, i.v., n = 10
2. Saline treated group-2: saline 0.05 ml/mouse/5 days/week, i.t., n = 10
3. SEQ ID NO:1 treated group: 1mg/kg/48 hours in 0.1 ml saline, i.v. n =10
4. SEQ ID NO:1 treated group: 2.5 mg/kg/48 hours in 0.1 ml saline, i.v., n =10
5. Intron A 10^3 unit /5days/week treated group: 10^3 unit Intron A in 0.05ml saline, i.t., n=10
6. Intron A 10^5 unit /5days/week treated group: 10^5 unit Intron A in 0.05ml saline, i.t., n=10
7. SEQ ID NO:1 1 mg/kg Plus Intron A 10^3 treated group: "3 + 5", n=10
8. SEQ ID NO:1 1 mg/kg Plus Intron A 10^5 treated group: "3 + 6", n=10
9. SEQ ID NO:1 2.5 mg/kg Plus Intron A 10^3 treated group: "4 + 5", n=10
10. SEQ ID NO:1 2.5mg/kg Plus Intron A 10^5 treated group: "4 + 6", n=10

Mice received 25 treatments with SEQ ID NO:1 and received 30 treatments with Intron A (treatment with Intron A for 3 consecutive weeks, 1 week off, and treatment with Intron A again for another 3 consecutive weeks).

Endpoints: The effects of SEQ ID NO:1 or Intron A, a standard immunotherapeutic for renal carcinoma, alone or in combination on tumor growth was determined. Tumor sizes were measured every week from day 7 after the tumor cell inoculation in two dimensions using a caliper, and the volume was expressed in mm^3 using the formula: $V = 0.5\ a \times b^2$, where a and b are the long and short diameters of the tumor, respectively. Mean tumor volumes calculated from each measurement were then plotted in a standard graph to compare the anti-tumor efficacy to that of controls. A day after the last treatment, tumors were excised from the animals and their weights were measured. A standard bar graph was used to demonstrate the differences in tumor weights with each bar representing mean tumor weight calculated from 10 animals.

The results of the tumor volume at different time points after tumor inoculation are shown in Figure 20A and the tumor weights at the experimental endpoint are shown in Figure 20B & 20C, illustrating that SEQ ID NO:1 and Intron A have significant anti-tumor efficacy as monotherapies and when in combination, the effect is at least additive. At sub-optimal drug dosages the combination effect remains at least additive. The results from the combination treatments of SEQ ID NO:1 and Intron A at high dose combination (2.5 mg/kg and 10^5, respectively) demonstrated dramatic regression of renal cell tumors in mice. A total of 5 mice in this group demonstrated

total regression and the tumors in the other 5 treated animals did not grow beyond 100 mg, showing partial regression and stabilization.

EXAMPLE 5: *In vivo* Testing of SEQ ID NO: 1 Alone and in Combination with Interferon Alpha in a Mouse Xenograft Model of Human Renal Carcinoma (Caki-1)

A second independent *in vivo* study was conducted as described above utilizing SCID mice (female, 6 weeks old) and Caki-1 (human renal carcinoma cell line) to test the efficacy of SEQ ID NO:1 alone or in combination with IFN alpha in a mouse xenograft model of human renal carcinoma. Briefly, 5 x 10^6 Caki-1 human renal carcinoma cells in 0.1 ml of PBS were injected subcutaneously at the right flank of the mice to induce tumor growth. Treatments started on the seventh day after tumor cell injection. Mice received a total of 23 treatments with SEQ ID NO:1 and 35 treatments with Intron A. Routes of administration are indicated as follows: intravenous (i.v.) and intratumoral (i.t).

Groups and treatment:

1. Saline treated group-A: saline 0.1ml/mouse/every 2 days, i.v., n = 10
2. Saline treated group-B: saline 0.05 ml/mouse/5 days/week, i.t., n = 10.
3. SEQ ID NO:1 treated group: 2.5mg/kg/every 2 days in 0.1 ml saline, i.v. n =10
4. GTI2040 treated group: 5 mg/kg/every 2 days in 0.1 ml saline, i.v., n =10
5. Intron 10^5 unit /5days/week treated group: 10^5 unit Intron in 0.05ml saline, i.t., n=10
6. SEQ ID NO:1 2.5 mg/kg Plus Intron10^5 treated group: **"3. + 5."**, n=10
7. SEQ ID NO:1 5 mg/kg Plus Intron10^5 treated group: **"4. + 5."**, n=10

The results of the tumor volume at different time points after tumor inoculation are shown in Figure 21A and the tumor weights at the experimental endpoint are shown in Figure 21B, illustrating that SEQ ID NO:1 and Intron A have significant anti-tumor efficacy as monotherapies and in combination the effect is at least additive.

EXAMPLE 6: *In vivo* Testing of SEQ ID NO: 1 Alone and in Combination with Interferon Alpha in a Mouse Xenograft Model of Human Renal Carcinoma (Caki-1)

A third independent *in vivo* study was conducted as described above utilizing SCID mice (female, 6 weeks old) and Caki-1 (human renal carcinoma cell line) to test the efficacy of SEQ ID NO:1 alone or in combination with IFN alpha in a mouse xenograft model of human renal carcinoma. Briefly, 5 x 10^6 Caki-1 human renal carcinoma cells in 0.1 ml of PBS were injected subcutaneously at the right flank of the mice to induce tumor growth. Treatments started on the seventh day after tumor cell injection. Mice received a total of 23 treatments with SEQ ID NO:1 and 35 treatments with Intron A. Routes of administration are indicated as follows: intravenous (i.v) and intratumoral (i.t.).

Groups and treatment:

1. Saline treated group: saline 0.1ml/mouse/every 2 days, i.v., n = 10
2. SEQ ID NO:1 treated group: 2.5mg/kg/every 2 days in 0.1 ml saline, i.v. n =10
3. SEQ ID NO:1-Scramble [SEQ ID NO:3] treated group: 2.5mg/kg/every 2 days in 0.1 ml saline, i.v. n =10
4. SEQ ID NO:1 plus Intron A 10^5 treated group: **"2. + 5."**, n =10
5. Intron A 10^5 unit /5days/week treated group: 10^5 unit Intron A in 0.05ml saline, i.t., n=10
6. SEQ ID NO:1-Scramble [SEQ ID NO:3] Plus Intron A 10^5 treated group: **"3. + 5."**, n=10

The results of the tumor volume at different time points after tumor inoculation are shown in Figure 22A and the tumor weights at the experimental endpoint are shown in Figure 22B, illustrating that both SEQ ID NO:1 and Intron A have significant anti-tumor efficacy as monotherapies and in combination the effect is at least additive. The scrambled control oligonucleotide, SEQ ID NO:1-scr [SEQ ID NO:3] had no efficacy as a monotherapy and did not improve the efficacy of Intron A.

EXAMPLE 7: *In vivo* Testing of SEQ ID NO: 1 Alone and in Combination with Interferon Alpha in a Mouse Xenograft Model of Human Renal Carcinoma (Caki-1)

A fourth independent *in vivo* study was conducted as described above utilizing SCID mice and Caki-1 (human renal carcinoma cell line) to test the efficacy of SEQ ID NO:1 alone or in combination with IFN alpha in a mouse xenograft model of human renal carcinoma (ten mice per group). IFN alpha is currently being tested in numerous clinical trials and is typically

administered subcutaneously. The effectiveness of SEQ ID NO:1 in combination with IFN alpha administered subcutaneously or intratumorally was examined in this mouse model of human renal carcinoma. Routes of administration are as follows: intravenous (i.v.), subcutaneous (s.c.) and intratumoral (i.t.).

Groups and treatments:

1. saline
2. SEQ ID NO:1 2.5 mg/kg/48 hrs; i.v.
3. IFN 100000 units, three days per week, i.t.
4. Combination of groups 2+3
5. IFN 100000 units, three days per week, s.c. opposite flank from tumor.
6. Combination of groups 2+5

The results of the tumor volume at different time points after tumor inoculation are shown in Figure 23. It is clear that both subcutaneous and intratumoral administration of Intron A are equally effective in combination with SEQ ID NO:1.

As SCID mice are immunocompromised, an alternative model that can be used to examine the efficacy of interferon alpha administration in combination with SEQ ID NO:1 in the treatment of renal carcinoma is the RenCa model of murine renal cancer in normal mice (for example, immune competent Balb/c mice). Although this model utilizes syngeneic RenCa cells, the target sequence in R2 for SEQ ID NO:1 is conserved in mice and as such the efficacy of SEQ ID NO:1 alone or in combination with IFN alpha can be tested in the RenCa murine tumor model. This model can also be used to test other immunotherapeutics, including various interleukinns, in combination with SEQ ID NO:1.

EXAMPLE 8: *In vivo* Testing of SEQ ID NO: 1 Alone and in Combination with Interferon Alpha in a Mouse Xenograft Model of Human Renal Carcinoma (A498)

Cell line: Human renal carcinoma cell line (A498) was grown as monolayer culture in Minimum essential medium (α-MEM) supplemented with 10% fetal bovine serum (FBS), 0.1 mM non-essential amino acid, 1.0 mM sodium pyruvate, 100 U/ml penicillin, 100 µg/ml streptomycin and

0.25 μg/ml amphotericin B and 2mM L-alanyl-l-glutamine at 37°C in an atmosphere of 5% CO_2 in air. The tumor cells were routinely subcultured twice weekly by trypsin-EDTA treatment. The cells were harvested from subconfluent logarithmically growing culture by treatment with trypsin-EDTA and counted for tumor inoculation.

Tumor Inoculation: An acclimation period of at least 7 days was allowed between animal receipt and commencement of tumor inoculation. When the female SCID mice were 6-7 weeks of age (20-25 g), each mouse was subcutaneously injected with $9x10^6$ A498 human renal carcinoma cells in 0.1 ml of PBS at the right flank of the mice to induce tumor growth.

Dose Preparation and Treatment: The dose for SEQ ID NO:1 was formulated using the following procedure. From a concentrated stock solution (usually prepared in ddH_2O at a concentration of 50 mg/ml and kept at -80°C), SEQ ID NO:1 was diluted with saline to achieve a final target concentration of 1 or 2.5 mg/ml on the first day of dosing. Enough SEQ ID NO:1 was diluted so that the compound could be administered by bolus infusion into the tail vein every other day for the duration of the experiment. A standard immunotherapeutic agent for renal carcinoma was utilized, Intron A™ (recombinant interferon alfa-2b). The dose and treatment schedules for Intron A are described below. The treatments were started 20 days after the tumor cell inoculation when the tumor size reached an approximate volume of 200 mm^3. Each treatment group contained 10 tumor-bearing mice. Routes of administration are as follows: intravenous (i.v.) and intratumoral (i.t.).

Groups and Treatments: The following treatment groups were evaluated in this experiment:

1. Saline treated group-1: saline 0.1ml/mouse/48 hours, i.v., n = 10
2. Saline treated group-2: saline 0.05 ml/mouse/5 days/week, i.t., n = 10
3. SEQ ID NO:1 treated group: 1mg/kg/48 hours in 0.1 ml saline, i.v. n =10
4. SEQ ID NO:1 treated group: 2.5 mg/kg/48 hours in 0.1 ml saline, i.v., n =10
5. Intron A 10^3 unit /5days/week treated group: 10^3 unit Intron A in 0.05ml saline, i.t., n=10
6. Intron A 10^5 unit /5days/week treated group: 10^5 unit Intron A in 0.05ml saline, i.t., n=10
7. SEQ ID NO:1 1 mg/kg Plus Intron A 10^3 treated group: "3 + 5", n=10
8. SEQ ID NO:1 1 mg/kg Plus Intron A 10^5 treated group: "3 + 6", n=10
9. SEQ ID NO:1 2.5 mg/kg Plus Intron A 10^3 treated group: "4 + 5", n=10

10. SEQ ID NO:1 2.5mg/kg Plus Intron A 10^5 treated group: "4 + 6", n=10

Treatment started from day 20 after inoculation of human renal tumor cells. Mice received 15 treatment with SEQ ID NO:1 and received 12 treatments with Intron A (treatment with Intron A for 2 consecutive weeks plus treatment for 2 additional days and off for the rest of the experiment).

Endpoints: The effects of SEQ ID NO:1 or Intron A, a standard immunotherapeutic for renal carcinoma, alone or in combination, on tumor growth was determined. Tumor sizes were measured every week from day 20 after the tumor cell inoculation in two dimensions using a caliper, and the volume was expressed in mm^3 using the formula: $V = 0.5\ a \times b^2$, where a and b are the long and short diameters of the tumor, respectively. Mean tumor volumes calculated from each measurement were then plotted in a standard graph to compare the anti-tumor efficacy to that of controls. A day after the last treatment, tumors were excised from the animals and their weights were measured. A standard bar graph was used to demonstrate the differences in tumor weights with each bar representing mean tumor weight calculated from 10 animals.

The results of the tumor volume at different time points after tumor inoculation are shown in Figure 24A and the tumor weights at the experimental endpoint are shown in Figure 24B & 24C, illustrating that both SEQ ID NO:1 and Intron A have significant anti-tumor efficacy as monotherapies and in combination, the effect is at least additive. At sub-optimal drug dosages the combination effect remains at least additive.

The results from the combination treatments of SEQ ID NO:1 and Intron A at high dose combination (2.5 mg/kg and 10^5, respectively) demonstrated dramatic regression of renal cell tumors in mice. All 10 mice in this group demonstrated total regression of all tumors.

EXAMPLE 9: *In vivo* Testing of SEQ ID NO: 1 Alone and in Combination with Interferon Alpha in a Mouse Xenograft Model of Human Renal Carcinoma (A498)

A second independent *in vivo* study was conducted as described above utilizing SCID mice (female, 6 weeks old) and A498 cell line (human renal carcinoma) to test the efficacy of SEQ ID NO:1 alone or in combination with IFN alpha in a mouse xenograft model of human renal

carcinoma. Briefly, 9 x 10^6 Caki-1 human renal carcinoma cells in 0.1 ml of PBS were injected subcutaneously at the right flank of the mice to induce tumor growth. Treatments started from day 20 after tumor cell injection. Routes of administration are as follows: intravenous (i.v.) and intratumoral (i.t.).

Groups and Treatments:

Group 1: treated with 0.1 ml saline per 48 hours, i.v. (n=10)

Group 2: treated with 50 ul saline per day/week x 5, i.t. (n=10)

Group 3: treated with 10000 units Intron A/mouse/dayx5/week for 2 weeks in 50 ul saline, i.t. (n=10)

Group 4: treated with 2.5 mg/kg/2ds SEQ ID NO:1 in 0.1 ml saline, i.v. (n=10)

Group 5: treated with 2.5mg/kg/2ds SEQ ID NO:1, i.v. plus 10000 units Intron A/ mouse /5ds/week for 2 weeks, i.t. (n=10).

Treatment schedules and dosages of SEQ ID NO:1 and Intron A and their combinations were performed as described above.

The results of the tumor volume at different time points after tumor inoculation are shown in Figure 25A and the tumor weights at the experimental endpoint are shown in Figure 25B, illustrating that both SEQ ID NO:1 and Intron A have significant anti-tumor efficacy as monotherapies and in combination the effect is at least additive.

EXAMPLE 10: *In vivo* Testing of SEQ ID NO: 1 Alone and in Combination with Interleukin-2 in a Mouse Xenograft Model of Human Renal Carcinoma (Caki-1)

Cell line: Human renal carcinoma cell line (Caki-1) was grown as monolayer culture in Minimum essential medium (α-MEM) supplemented with 10% fetal bovine serum (FBS), 0.1 mM non-essential amino acid, 1.0 mM sodium pyruvate, 100 U/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B and 2mM L-alanyl-l-glutamine at 37°C in an atmosphere of 5% CO_2 in air. The tumor cells were routinely subcultured twice weekly by trypsin-EDTA treatment. The cells were harvested from subconfluent logarithmically growing culture by treatment with trypsin-EDTA and counted for tumor inoculation.

Tumor Inoculation: An acclimation period of at least 7 days was allowed between animal receipt and commencement of tumor inoculation. When the female SCID mice were 6-7 weeks of age (20-25 g), each mouse was subcutaneously injected with $1x10^7$Caki-1 human renal carcinoma cells in 0.1 ml of PBS at the right flank of the mice to induce tumor growth.

Dose Preparation and Treatment: The dose for antisense oligonucleotide drug (SEQ ID NO:1) was formulated using the following procedure. From a concentrated stock solution (usually prepared in ddH_2O at a concentration of 50 mg/ml and kept at -80°C), SEQ ID NO:1 was diluted with saline to achieve a final target concentration of 10 mg/ml on the first day of dosing. Enough SEQ ID NO:1 was diluted so that the compound could be administered by bolus infusion into the tail vein every other day for the duration of the experiment. The treatments were started 7 days after the tumor cell inoculation when the tumor size reached an approximate volume of 100 mm^3. Each treatment group contained 10 tumor-bearing mice. The dose and treatment schedules for IL2 are described below. Routes of administration are as follows: intravenous (i.v.) and intraperitoneal (i.p.).

Groups and treatments: The following treatments were evaluated in this experiment:

1. Saline treated group: saline 0.1ml/mouse/48 hours, i. v. n = 10
2. SEQ ID NO:1 treated group: 10 mg/kg/48 hours in 0.1 ml saline, i. v., n =10 (17 treatments total)
3. IL2 treatment cycle: 8 days (treated 4days then stop 2 days then treated another 4 days)
   I-High Dose (20000 unit)/2 times for one day treatment, i .p. n=10
   II-Low Dose (5000 unit)/2 times for one day treatment, i .p. n=10
4. SEQ ID NO:1 + IL-2 treated group-I: n=10
5. SEQ ID NO:1 + IL-2 treated group-II: n=10

Endpoints: The anti-tumor effects of SEQ ID NO:1 and IL-2, a standard immunotherapeutic for renal carcinoma, alone or in combination, on tumor growth, was determined. Tumor sizes were measured every week day from day 7 after the tumor cell inoculation in two dimensions using a caliper, and the volume was expressed in mm^3 using the formula: $V = 0.5\ a \times b^2$, where a and b are the long and short diameters of the tumor, respectively. Mean tumor volumes calculated from each measurement were then plotted in a standard graph to compare the anti-tumor efficacy to that of controls. A day after the last treatment, tumors were excised from the animals and their

weights were measured. A standard bar graph was used to demonstrate the differences in tumor weights with each bar representing mean tumor weight calculated from 10 animals.

The results of the tumor volume at different time points after tumor inoculation are shown in Figure 26A and the tumor weights at the experimental endpoint are shown in Figure 26B, illustrating that both SEQ ID NO:1 and IL-2 have anti-tumor efficacy as monotherapies but that SEQ ID NO:1 is superior to both high and low dose IL-2 therapy. In combination the effect is at least additive.

The results from the combination treatments of SEQ ID NO:1 and IL-2 at both low and high dose combination (5000U and 20000U, respectively) demonstrated dramatic regression of renal cell tumors in mice. All 10 mice in each group demonstrated total regression of all tumors.

EXAMPLE 11: Clinical Trials

Examples of potential designs for clinical trials to test SEQ ID NO:1 in combination with various known chemotherapeutics are provided in Table 6.

Examples of ongoing clinical trials and other clinical trials that have been approved by the NCI using SEQ ID NO: 1 are outlined below. Details of the protocols involved for each of trials 1-7 are provided in Table 7. The following describes the Protocols involved for each trial:

1. PROTOCOL LO1-1409 (RENAL CELL CARCINOMA)
Study Description:
SEQ ID NO:1 and capecitabine combination therapy in patients with advanced or metastatic renal cell carcinoma (Phase I/II)
Population: Advanced or metastatic renal cell carcinoma having failed standard therapy
Study regimen: SEQ ID NO:1 (CIV infusion)
+ capecitabine
cycles: 21 days + 7 days rest
Phase I / II
Status: Ongoing in Phase II

Dosing: SEQ ID NO:1 was administered as a continuous intravenous infusion for 21 days at a starting dose of 148.0 mg/m^2/day in combination with capecitabine administered orally at a fixed dose of 1660 mg/m^2/day (divided into two daily doses for 21 days) followed by 7 days of rest.

2. PROTOCOL L6093 (BREAST)

Study Description

A Phase II Study of SEQ ID NO: 1 and Capecitabine in the treatment of Metastatic Breast Cancer

Population: Breast cancer, metastatic and failing 2 or more prior regimens

Study regimen; SEQ ID NO: 1 + Capecitabine
14 days in 21 day cycle

Subjects: 40 (2 Stages: 20 ea)

Phase II

Status: Ongoing study

3. PROTOCOL L6104 (NSCLC)

Study Description

A Phase I/II Trial of SEQ ID NO: 1 and Docetaxel in Metastatic or Advanced Non-Small Cell Lung Cancer

Population: Metastatic or unresectable locally advanced NSCLC

Study regimen SEQ ID NO: 1+ Docetaxel

Subjects: 42 (12 Phase I; 30 Phase II)

Status: Ongoing study

4. PROTOCOL L6090 (SOLID TUMOURS)

Study Description

A Phase I Study of SEQ ID NO: 1 and Gemcitabine in Patients with Solid Tumours

Population: Solid tumours metastatic or unresectable and for which curative or palliative measures do not exist or are no longer effective.

Study regimen SEQ ID NO:1 + Gemcitabine

Subjects: 34

Status: Ongoing study

5. PROTOCOL L6108 (AML)

Study Description

A Phase I Trial of SEQ ID NO:1 in combination with high-dose cytarabine in refractory or relapsed acute myeloid leukaemia (AML)

Population: Acute myeloid leukaemia refractory or relapsed.

Study regimen SEQ ID NO:1 + cytarabine

Subjects: 30

Status: Ongoing study

6. PROTOCOL L6099 (COLORECTAL)

Study Description

A Phase I Trial of SEQ ID NO: 1 , Oxaliplatin and Capecitabine in Refractory Unresectable Colorectal Cancer

Population: Locally advanced or metastatic colorectal cancer (refractory, unresectable). Patients must have had at least one standard prior chemotherapy with no prior oxaliplatin-containing regimen.

Study regimen SEQ ID NO: 1 + oxaliplatin & capecitabine

Subjects: 15-20

Phase I

Status: Ongoing study

7. PROTOCOL L6102 (PROSTATE)

Study Description

A Phase II Study of SEQ ID NO: 1 and Docetaxel in Patients with Hormone-Refractory Prostate Cancer

Population: Patients with hormone-refractory prostate cancer and rising PSA levels (PSA≥20).

ECOG 0-2, with adequate organ function

Study regimen SEQ ID NO: 1 + Docetaxel

Subjects: 40

Phase II

Status: awaiting Phase II dose from ongoing trial with same drug combination.

TABLE 6: Examples of Clinical Trials that can be Conducted using Antisense Oligonucleotide SEQ ID NO:1 in Combination with Various Chemotherapeutic Agents

Disease	Performance Status (PS) and Organ Function	Prior Therapy	Trial Design Schedule
Solid tumours	PS ≤ 2** Must have adequate hematologic, renal, and metabolic function	No limit stated $\geq$ 4 wks since prior RT $\geq$ 3 wks since prior therapy (6 wks for nitrosourea or mitomycin C)	**SEQ ID NO:1:** CIV day 1-21 148-185 mg/m^2/day **Capecitabine:** PO BID day 1-21 500-1500 mg 28-day cycle
Solid tumours	PS 0-2** Creatinine: < 2.0 mg/dL SGOT: < 2 x normal Bilirubin: < 1.5 mg/dL WBC: > 4000 PLT: > 100,000	No limit stated $\geq$ 4 wks since prior tx	**SEQ ID NO:1:** CIV day 1-21 100-185 mg/m^2/day **Capecitabine:** PO BID day 1-21 1100-2000 mg/m^2/day 28-day cycle
Breast	PS 60-100%** Must have adequate hematologic, renal, and hepatic function	$\geq$ 2 prior regimens No prior capecitabine or 5-FU unless in adjuvant setting	**SEQ ID NO:1:** CIV day 1-21 74-185 mg/m^2/day **Capecitabine:** PO BID day 8-21

Disease	Performance Status (PS) and Organ Function	Prior Therapy	Trial Design Schedule
			600-1000 mg/m^2 28-day cycle
Colorectal	PS 0-2** bilirubin ≤ 1.5 x ULN SGOT/SGPT ≤ 3 x ULN	1 prior CT or adjuvant	**SEQ ID NO:1:** CIV day 1-21 185 mg/m^2/day **Capecitabine:** PO BID day 1-14 start at 850 mg/m^2 28-day cycle
Colorectal	PS 60-100%** Must have adequate hematologic, renal, and hepatic function	No prior oxaliplatin	**SEQ ID NO:1:** CIV day 1-21 74-185 mg/m^2/day **Capecitabine:** PO BID d2-15 600-1000 mg/m^2 **Oxaliplatin:** IV day 2 130 mg/m^2/day 28-day cycle
Solid tumours	PS 0-2** Abnormal organ function permitted	≥ 1 prior regimens No prior docetaxel	**SEQ ID NO:1:** CIV day 1-21 50-185 mg/m^2/day

Disease	Performance Status (PS) and Organ Function	Prior Therapy	Trial Design Schedule
			Docetaxel: IV weekly x 3 30-35 mg/m^2 28-day cycle
Solid tumours	PS 0-1** Abnormal organ function not permitted	No restrictions	**SEQ ID NO:1:** CIV day 1-21 148-185 mg/m^2/day **Docetaxel:** IV day 1, 8, 15 30-35 mg/m^2 28-day cycle
NSCLC & other solid tumours	PS 0-1** Abnormal organ function not permitted	≤ 2 prior CT	**SEQ ID NO:1:** CIV day 1-22 1-5 mg/kg/day **Docetaxel:** IV day 8, 15, 22 35 mg/m^2 28-day cycle
NSCLC	PS 0-2** Must have adequate hematological, renal, & hepatic function	Prior platinum-based CT required No prior taxane CT > 1 systemic tx not permitted	**SEQ ID NO:1:** CIV day 1-14 74-185 mg/m^2/day **Docetaxel:**

Disease	Performance Status (PS) and Organ Function	Prior Therapy	Trial Design Schedule
		≥ 28 days since prior surgery or RT	IV day 3 1st cycle, day 1 2nd cycle 60-75 mg/m^2/day 21-day cycle
Prostate	PS 0-2** Abnormal organ function not permitted	No prior CT Must have failed front-line hormonal treatment	**SEQ ID NO:1:** CIV day 1-21 3-5 mg/kg/day **Docetaxel:** IV day 1, 8, 15 30-36 mg/ m^2/day 28-day cycle
Genitourinary	PS 0-2** creatinine 2X ULN, liver function <1.5 ULN	3 prior CT	**SEQ ID NO:1:** CIV day 1-21 100-185 mg/m^2/day **Docetaxel:** IV 40-80 mg/m^2 every 3 wks
Solid tumours	PS 0-2** Abnormal organ function not permitted	≤ 2 prior tx	***Schedule A:*** **SEQ ID NO:1:** CIV day 1-21 90-190, RP2D mg/m^2/day

Disease	Performance Status (PS) and Organ Function	Prior Therapy	Trial Design Schedule
			Gemcitabine: IV day 1, 8, 15 1000-1200 mg/m^2 ***Schedule B:*** **SEQ ID NO:1:** CIV day 1-21 (RP2D) **Gemcitabine:** CIV day 1, 8, 15 750-1200 mg/m^2 28-day cycles
Solid tumours	PS 0-2** Abnormal organ function not permitted	No restrictions	**SEQ ID NO:1:** CIV day 2-21 cycle 1 (100-185 mg/m^2) CIV day 1-21 for subsequent cycles **Gemcitabine:** IV day 1, 8, 15 400-1000 mg/m^2 28-day cycle
Colon cancer	PS $\geq$ 60%** survival $\geq$ 3 mo	Must have progressed following 5-FU, irinotecan, & oxaliplatin CT	**SEQ ID NO:1:** CIV day 1-21 85-185 mg/m^2/day

Disease	Performance Status (PS) and Organ Function	Prior Therapy	Trial Design Schedule
	Must have adequate hematologic, renal, and hepatic function		**Gemcitabine:** IV day 1, 8 every 6 wks 1000 mg/m^2 **Capecitabine:** PO BID d1-14 every 3 wks 650 mg/m^2
NSCLC	PS > 60%** survival > 3 mo Must have adequate hematologic, renal, and hepatic function	No prior gemcitabine or SEQ ID NO:1 ≥ 4 wks since prior CT ≥ 6 wks since prior mitomycin C CT ≥ 2 wks since prior RT	**SEQ ID NO:1:** CIV day 1-21 74-185 mg/m^2/day **Gemcitabine:** IV day 2, 9, 16 800-1000 mg/m^2 28-day cycle
Renal cell carcinoma	PS 0-2** Must have adequate hematologic, renal, and hepatic function	**Phase 1** No prior gemcitabine CT **Phase 2** ≤ 2 prior tx allowed ≥ 4 wks since prior RT, surgery, or tx	**SEQ ID NO:1:** CIV day 1-21 111-185 mg/m^2/day **Gemcitabine:** IV weekly x 3 800-1000 mg/m^2 28-day cycle

Disease	Performance Status (PS) and Organ Function	Prior Therapy	Trial Design Schedule
Breast cancer	PS 60-100%** Must have adequate hematologic, renal, and hepatic function	≤ 1 prior tx no prior platinum or gemcitabine allowed	**SEQ ID NO:1:** CIV day 1-21 74-185 mg/m^2/day **Gemcitabine:** IV day 2, 9 600-1000 mg/m^2/day **Oxaliplatin:** IV day 2 130 mg/m^2/day 28-day cycle
AML	PS 0-2** Abnormal organ function permitted: Cr ≤ 2.0 mg/dL total bilirubin < 2.0 mg/dL AST/ALT < 5 x ULN	Prior therapy O.K.	**SEQ ID NO:1:** CIV day 1-21 120-280 mg/m^2/day **Idarubicin:** IV day x3 12 mg/m^2/day
AML	PS 0-2** Abnormal organ function permitted: Cr ≤ 2.0 mg/dL total bilirubin ≤ 2.0 mg/dL	Prior therapy O.K.	**SEQ ID NO:1:** CIV day 1-21 120-280 mg/m^2/day **Ara-C:** IV day x4 1 g/m^2/day

Disease	Performance Status (PS) and Organ Function	Prior Therapy	Trial Design Schedule
AML, CML	PS 0-2** Cr < 2 bilirubin < 2	At least 1 prior therapy	**SEQ ID NO:1:** CIV day 1-5 2-10 mg/kg/day **Ara-C:** IV day 1-5 1 g/m^2/day
AML	PS 0-2** Abnormal organ function not permitted	Chemotx, including auto or allo SCT	**SEQ ID NO:1:** CIV day 1-8 3.5, 5 mg/kg/day **Mitoxantrone:** IV day 4-8 6 mg/m^2/day **Etoposide:** IV day 4-8 80 mg/m^2/day **Ara-C:** IV day 4-8 500-1000 mg/m^2/day
CML	PS 0-2** Abnormal organ function permitted: Cr $\leq$ 2.0 mg/dL	Imatinib mesylate failed	**SEQ ID NO:1:** CIV day 1-21 120-280 mg/m^2/day **Fludarabine:**

Disease	Performance Status (PS) and Organ Function	Prior Therapy	Trial Design Schedule
	total bilirubin < 2.0 mg/dL AST/ALT < 5 x ULN		IV day 3-7 30 mg/m^2/day **Ara-C:** IV day 3-7 2 g/m^2/day **Filgrastim:** 5 mcg/kg/day, start day 8
Metastatic cancer	ECOG ≤ 2 Must have adequate hematologic, renal, and hepatic function	≥ 4 wks since prior RT **Phase 1**: unlimited chemotherapy **Phase 2**: must have prior paclitaxel and carboplatin	**SEQ ID NO:1:** CIV day 1-14 125-185 mg/m^2/day **Carboplatin:** AUC = 5, 6 **Paclitaxel:** IV weekly 135-175 mg/m^2 Cycles repeat every 21 days
Head & Neck, esophagus, lung	PS 0-2** Abnormal organ function not permitted	One prior multimodality tx permitted (including platinum-based)	**SEQ ID NO:1:** CIV day 1-21 (dose TBD) **Cisplatin:** IV weekly

Disease	Performance Status (PS) and Organ Function	Prior Therapy	Trial Design Schedule
			15-40 mg/ m^2 28-day cycle
SCLC	PS 0-2** Abnormal organ function not permitted	No prior chemotx	**SEQ ID NO:1:** CIV day 1-21 3- 5 mg/kg/day **Cisplatin:** IV day 1 50-60 mg/m^2/day **Irinotecan:** IV day 1, 8, 15 50-60 mg/m^2/day
Pancreatic adenocarc	PS 0-2** Abnormal organ function not permitted	Prior gemcitabine required ≥ 4 wks since gemcitabine regimen No prior irinotecan allowed	**SEQ ID NO:1:** CIV day 1-14 104-185 mg/m^2/day **Irinotecan:** IV day 1, 8 75-125 mg/m^2/day 21-day cycle
Pancreas, gall bladder, & biliary ducts	PS 0-2** Total bilirubin < 3X ULN eligible	1 prior tx: either gemcitabine or bryo/Taxol	**SEQ ID NO:1:** CIV day 1-21 (dose TBD) **5-FU:** CIV day 1-21

Disease	Performance Status (PS) and Organ Function	Prior Therapy	Trial Design Schedule
			100-225 mg/m^2/day 28-day cycle
Pancreas cancer	PS = ≥ 60%** AGC > 1,500/mcL Hgb ≥ 9 mg/dL plts > 100,000/mcL creat ≤ 1.5 ULN bilirubin ≤ 1.5 ULN ALP/SGOT/SGPT ≤ 3 x iULN	Must have PD after previous gemcitabine chemotx for metastatic pancreatic cancer	**SEQ ID NO:1:** CIV day 1-21 85-185 mg/m^2/day **Oxaliplatin:** IV day 1 130 mg/ m^2/day **Capecitabine:** PO BID d1-14 1000 mg/ m^2/day Cycles repeat every 21 days Schedule modified if significant toxicity in 2/3 patients @ dose level 1: **SEQ ID NO:1:** CIV day 1-14 85-185 mg/ m^2/day **Oxaliplatin and capecitabine**: as above

Disease	Performance Status (PS) and Organ Function	Prior Therapy	Trial Design Schedule
			Cycles repeat every 21 days
Prostate cancer	PS ≤ 2**	Hormone-refractory;	**SEQ ID NO:1:**
		no prior chemotx;	CIV day 1-14
	ANC ≥ 1.5 x 10e9/L	≥4 wks since prior XRT	111-185 mg/ m^2/day
	plts ≥ 100 x 10e9/L		**Mitoxantrone:**
	creat ≤ 2 x ULN or		IV every 3 weeks
	CrCl ≥40 mL/min		12 mg/m^2
	bili ≤1.5 x ULN		**Prednisone:**
	AST/ALT ≤3xULN		PO BID
	LVEF ≥50%		5 mg
			Cycles repeat every 21 days to 10 cycles or PD

* Abbreviations are as follows:

AGC: Absolute granulocyte count
Allo SCT: Allogenic stem cell transplantation
Auto SCT: Autologous stem cell transplantation
ALP: Alkaline phosphatase
ALT: Alanine aminotransferase
ANC: Absolute neutrophil count
AST: Aspartate aminotransferase
Bili: bilirubin
CIV: Continuous intravenous infusion
Cr and Creat: Creatinine
CT: Computerized axial tomography
Hgb: Haemoglobin
LVEF: Left ventricular ejection fraction
PLT and plts: Platelet count
PO BID: By mouth, 2 times a day
RT: Radiotherapy
Rx: Therapy
SGOT: Serum glutamic-oxalacetic transferase
SGPT: Serum glutamic pyruvic transaminase
Tx: Treatment
ULN: Upper limit of normal
WBC: White blood cell/ white blood count

** PS numerical values reflect ECOG status; percentage values reflect Karnofsky performance status

TABLE 7: Current Clinical Trials using Antisense Oligonucleotide SEQ ID NO:1 in Combination with Various Chemotherapeutic Agents

Protocol	Objectives	Drug Regimen
1. (1409)	To determine the recommended Phase II dose To evaluate the response rate To evaluate the toxicity To determine pharmacokinetic data	SEQ ID NO: 1 + Capecitabine SEQ ID NO:1 was administered as a continuous intravenous infusion for 21 days at a starting dose of 148.0 mg/m^2/day (phase I) or 185 mg/m^2/day (phase II) followed by 7 days of rest Capecitabine was administered orally at a fixed dose of 1660 mg/m^2/day (divided into two daily doses for 21 days) followed by 7 days of rest.
2. (L6093)	To evaluate the response rate and response duration To evaluate the toxicity To determine pharmacokinetic data To investigate potential markers of RNR inhibition and fluoropyrimidine metabolism.	SEQ ID NO:1 (148-185 mg/m2/day) + Capecitabine (600-1000 mg/m2 bid for 14 days). 21 day treatment cycle. SEQ ID NO:1 will be administered as a 14-day continuous IV infusion on days 1-15 with a starting dose of 74 mg/m2/day. The starting dose of capecitabine

Protocol	Objectives	Drug Regimen
		will be 600 mg/m2 orally bid on days 2-14. Patients will have one week rest and then on completion of the 21 day cycle, start day one of next cycle.
3. (L6104)	To determine the recommended Phase II dose To assess the objective tumour response rate To assess the toxicity, stable disease rate, time to disease progression, objective response duration and duration of stable disease. To investigate PK parameters To measure the baseline and post-treatment levels of RNR activity	SEQ ID NO:1 (2-5 mg/kg/day) + Docetaxel (60-75 mg/m2 IV) SEQ ID NO:1 continuous IV infusion day 1 to 14 every 21 days. Docetaxel IV day 3 in cycle 1, day 1 subsequent cycles, every 21 days. Dosage of SEQ ID NO:1 can be started at 3-5 mg/kg/day and be reduced to 2 mg/kg/day if a dose reduction is indicated.
4. (L6090)	Primary To determine the toxicity profile and MTD Secondary To examine PK and PD	SEQ ID NO:1 (100-185 mg/m2/day) + Gemcitabine (400-1000 mg/m2) In Cycle 1, the SEQ ID NO:1 CIV is given from day 2-16 every 28 days. Only from cycle

Protocol	Objectives	Drug Regimen
	To determine the effects on RNR R2 subunit mRNA and protein expression To examine the effects on apoptotic markers and cell cycle regulatory proteins and to analyze the serum biomarkers	2 onwards, SEQ ID NO:1 CIV is given from day 1-15 every 28 days. In Cycle 1, gemcitabine is given weekly on days 1, 8, and 15 every 28 days. Only from Cycle 2 onwards, gemcitabine is given weekly on days 2, 9, and 16 every 28 days.
5. (L6108)	To determine the MTD To document therapeutic responses To evaluate PK To measure R2 mRNA To assess apoptosis in leukemic cells To measure RNR enzymatic activity	*Cohort 1* SEQ ID NO:1 (3.5-5 mg/kg/day) + Cytarabine (2000-3000 mg/m2 q12 hours) SEQ ID NO:1 will be administered by continuous IV infusion for a total of 168 days (days 1 to 7). Cytarabine will be administered IV over 2 hours every 12 hours for a total of 12 doses (day 2 to 7) *Cohort 2* SEQ ID NO:1 (3.5-5 mg/kg/day) + Cytarabine (1500-2000 mg/m2 q12 hours)

Protocol	Objectives	Drug Regimen
		SEQ ID NO:1 will be administered by continuous IV infusion for a total of 144 hours (days 1 to 6). Cytarabine will be administered IV over 4 hours every day for 5 days (days 2 to 6) for a total of 5 doses.
6. (L6099)	Primary To establish maximum tolerated dose To describe the toxicities at each dose level studied. Secondary To evaluate the pharmacokinetics of the combination therapies. To evaluate levels of ribonucleotide reductase –M2 subunit (RR-M2) mRNA levels. To quantify changes in dCTIP levels in peripheral mononuclear cells as surrogate marker of RR inhibition.	SEQ ID NO:1 (3-5 mg/kg/day) + Oxaliplatin (130 mg/m2) & Capecitabine (600-1200 mg/m2/BID) The dose of oxaliplatin will be fixed at 130 mg/m2 and administered IV over 2 hours on day 2 of a 21 day treatment cycles. After the first cycle, oxaliplatin will be given on day 1. The starting dose of capecitabine will be 600 mg/m2 twice orally and will be given beginning on day 2 of the first cycle for 28 doses (14 days) and subsequently on day 1 after initiation of SEQ ID NO:1.

Protocol	Objectives	Drug Regimen
		SEQ ID NO:1 will be given as a continuous infusion through a central line over 14 days beginning on day 1 of treatment.
7. (L6102)	Primary To establish the efficacy using PSA-response rate Secondary To estimate objective tumour response To estimate the median time to progression To investigate safety and tolerability To estimate the median of duration of PSA-response To measure baseline and post-treatment levels of RNR activity	SEQ ID NO:1 (5 mg/kg/day) + Docetaxel (75 mg/m2) SEQ ID NO:1: Continuous IV infusion for 14 days of a 21-day cycle to start with a docetaxel bolus. Docetaxel: Administered IV every 21 days. For cycle 1 only administered on day 3. Administered on day 1 for subsequent cycles. Prednisone: 5 mg po bid continuously

Preliminary unaudited data for protocol 1409 showed that amongst the 25 response-evaluable patients at the phase II dose; 13 (52%) had stable disease (SD) as best response (median duration: 4 months, range 2-10), and 1 durable (8 months) partial response (PR) was observed. At the phase II dose, the patient with PR experienced a unidimensional tumor reduction of 39%, and the patient with the longest duration SD had a 23% tumor reduction. One additional patient at dose level 0 also had SD and a 13% decrease in tumor size. The combination of SEQ ID NO:1 and capecitabine is tolerated at the recommended phase II dose with expected toxicities. Treatment

has been well tolerated with few treatment-related toxicities other than those already known to occur with these drugs with acceptable frequency.

Clinical trials are under development to test the efficacy of SEQ ID NO:1 in combination with a cytokine, such as IFN-alpha or an interleukin, in the treatment of early stage renal cell carcinoma. The treatment will be a first-line therapy for newly diagnosed, previously untreated patients.

EXAMPLE 12: Efficacy of SEQ ID NO:1 Alone *in vivo* in Mouse Xenograft Models

It has been demonstrated previously using various mouse models of solid tumors, haematologic neoplasms and metastasis that treatment with SEQ ID NO:1 alone is effective in inhibiting the growth and metastasis of various tumor types (i.e. prolonging survival of mice with lymphoma or erythroleukemia). The results are summarized in Table 8.

Table 8: Summary of Effects of SEQ ID NO:1 on Tumour Growth and Metastasis

Assay	Tumour Placed in Mouse	Results
Tumour Growth	Mouse Fibrosarcoma[1]	• Inhibition of tumour volume in C3H mice by approximately 80% on Day 15 after tumour implantation • Inhibition of tumour weight by approximately 80% • Dose-dependent decrease in tumour weight in C3H mice at doses of 0.5 to 30 mg/kg
	Human Colon Adenocarcinoma	• Inhibition of tumour size in CD-1 nude mice by approximately 80% on Day 18 after tumour implantation • Inhibition of tumour weight by approximately 80% • Dose-dependent decrease in tumour weight in

Assay	Tumour Placed in Mouse	Results
		CD-1 nude mice at doses of 1.0 to 6.0 mg/kg
	Human Melanoma	• Inhibition of tumour size by approximately 80 to 85% on Days 31 after tumour implantation • Inhibition of tumour weight of approximately 80%
	Human Breast Adenocarcinoma	• Inhibition of tumour weight by approximately 80% on Day 31 after tumour implantation • Inhibition of tumour size by approximately 80%
	Human Pancreatic Adenocarcinoma	• Complete inhibition of tumour growth up to 39 days after tumour implantation • Inhibition of tumour weight by approximately 65%
	Human Ovary Adenocarcinoma	• Inhibition of tumour size by approximately 35% in Balb/c Nu-Nu mice on Days 19 to 25 after tumour implantation (Figure 11) Inhibition of tumour weight of approximately 50%
	Human Lung Carcinoma	• Inhibition of tumour size by approximately 85% in CD-1 Nude mice on Days 14 to 19 after tumour implantation • Inhibition of tumour weight of approximately 70%
	Human Liver Carcinoma	• Inhibition of tumour size by 45% in CD-1 nude mice on Day 30 after tumour implantation • Inhibition of tumour weight of approximately

Assay	Tumour Placed in Mouse	Results
		65%
	Human Glioblastoma-Astrocytoma	• Inhibition of tumour weight of approximately 65%
	Human Renal Carcinoma	• Approximately 90% inhibition of tumour size
	Human Renal Carcinoma[2]	• Approximately 97% inhibition of tumour size
	Human Renal Carcinoma[2]	• Complete regression of all tumours in treated mice
	Human Cervical Carcinoma[2]	• Inhibition of tumour size by approximately 90% in SCID mice on Day 22 after tumour implantation • Inhibition of tumour weight of approximately 90%
	Human Cervical Carcinoma[2]	• Inhibition of tumour size and weight by approximately 60% in SCID mice on Day 17 after tumour implantation
Metastasis	Mouse Fibrosarcoma (*ex vivo*)	• Decrease by approximately 65% in the number of tumour metastases to lungs
	Human Melanoma (*ex vivo* and *in vivo*)	• Decrease by approximately 95% in the number of tumour metastases to lungs
Prolonged	Burkitt's	• All untreated mice died by day 23

Assay	Tumour Placed in Mouse	Results
Survival	Lymphoma	• Treated mice survived beyond day 73 with the exception of one mouse that died at day 69 • Treatment prolonged survival
	Burkitt's Lymphoma	• All untreated mice died by day 20 • All treated mice survived to the end of the experimental period (140 days) • Treatment prolonged survival
	Erythroleukemia	• All untreated mice died within 36 days • Treated mice survived beyond day 71 except for one mouse which died at day 22 • Treatment prolonged survival

The demonstrated efficacy of SEQ ID NO:1 in such a range of situations indicates that it has potential application as part of a combination therapy with one or more immunotherapeutic agents in the treatment of a variety of cancers amenable to immunotherapy.

The disclosure of all patents, publications, including published patent applications, and database entries referenced in this specification are specifically incorporated by reference in their entirety to the same extent as if each such individual patent, publication, and database entry were specifically and individually indicated to be incorporated by reference.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A method of treating cancer in a mammal comprising administering to said mammal a combination comprising:
   (a) one or more antisense oligonucleotides comprising a sequence of at least 7 contiguous nucleotides that are complementary to portion of the mammalian ribonucleotide reductase R2 mRNA, and
   (b) one or more immunotherapeutic agents.

2. The method according to claim 1, wherein said antisense oligonucleotide comprises a sequence of at least least 7 contiguous nucleotides of SEQ ID NO:1.

3. The method according to claim 2, wherein said antisense oligonucleotide comprises SEQ ID NO:1.

4. The method according to claim 1, wherein said one or more immunotherapeutic agent is one or more cytokine.

5. The method according to claim 4, wherein said one or more cytokine is an interferon or an interleukin or a combination thereof.

6. The method according to claim 1, wherein said mammal is a human.

7. The method according to claim 6, wherein cancer is renal cancer.

8. The method according to claim 7, wherein said renal cancer is early stage renal cancer.

9. A method of treating early stage renal cancer in a human comprising administering to said human a combination comprising:
   (a) one or more antisense oligonucleotides comprising a sequence as set forth in SEQ ID NO:1, and
   (b) one or more cytokines.

ABSTRACT

Combinations comprising one or more antisense oligonucleotides against the gene encoding a mammalian ribonucleotide reductase R2 protein and one or more immunotherapeutic agents are provided. The combinations further comprise one or more chemotherapeutic agents. The combinations are more effective in decreasing the growth of neoplastic cells than either the antisense oligonucleotide or the immunotherapeutic agent(s) alone. Methods of treating cancer in a mammal using the combinations are also provided.

FIGURE 1

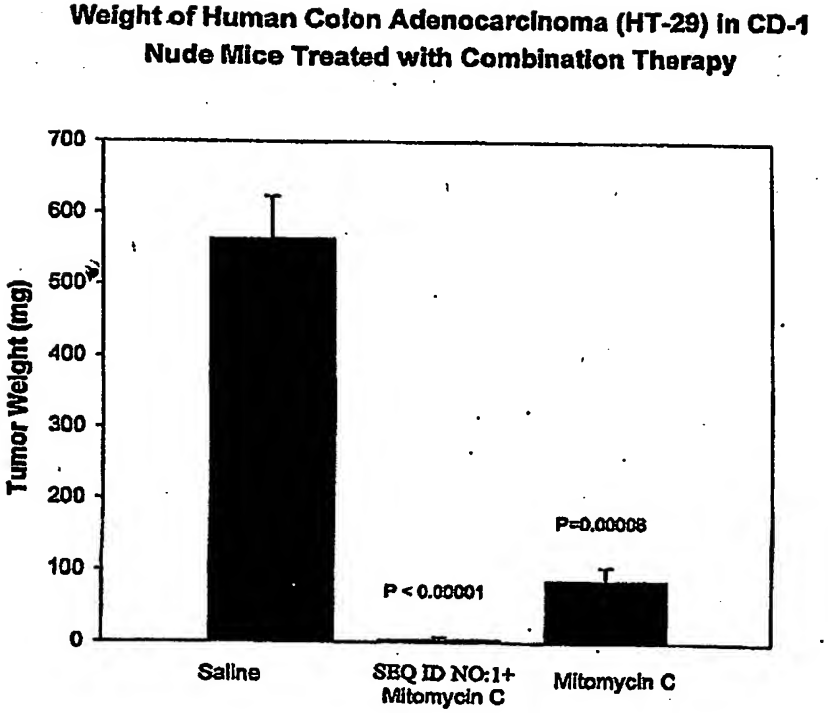

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FIGURE 2

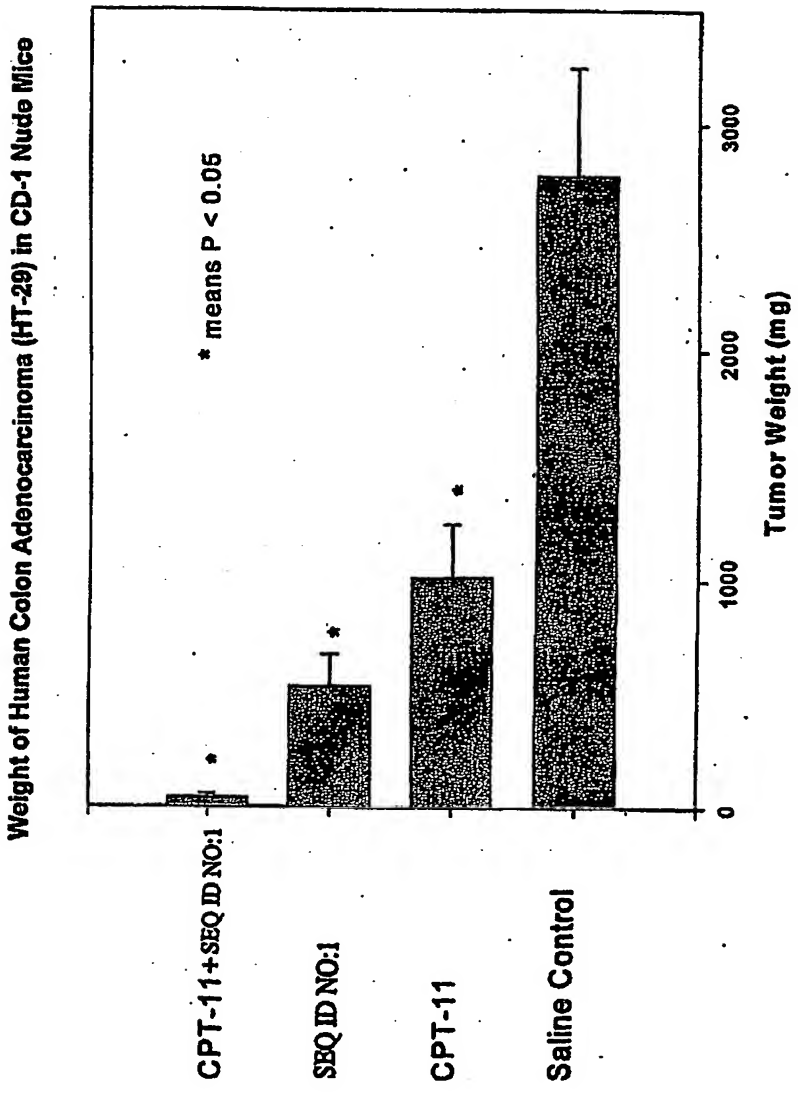
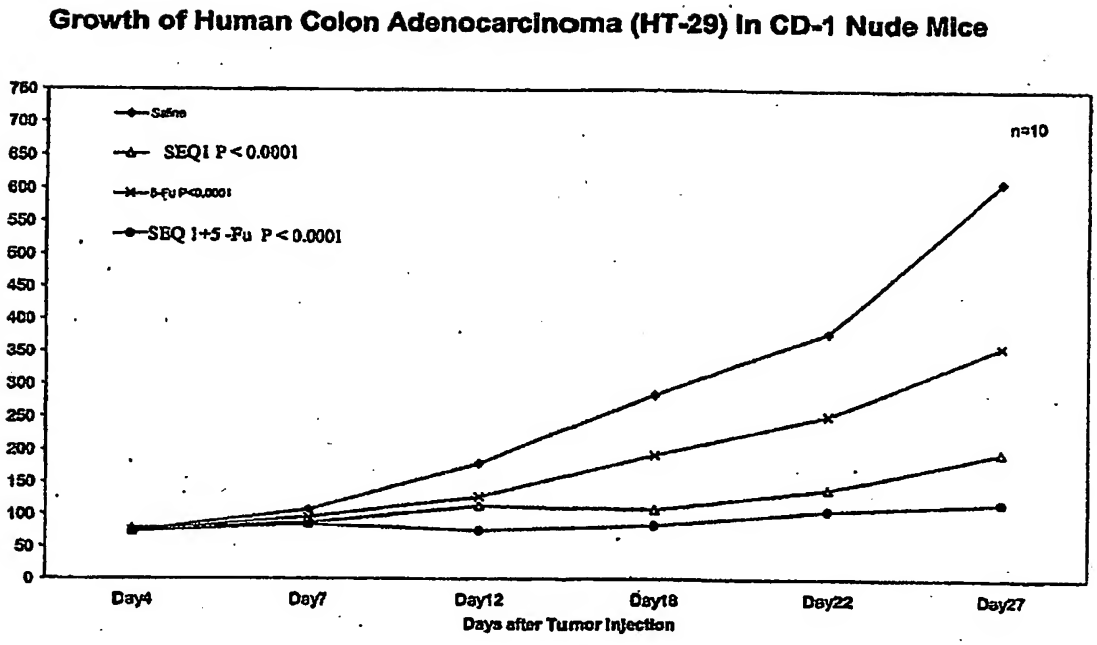


FIGURE 3



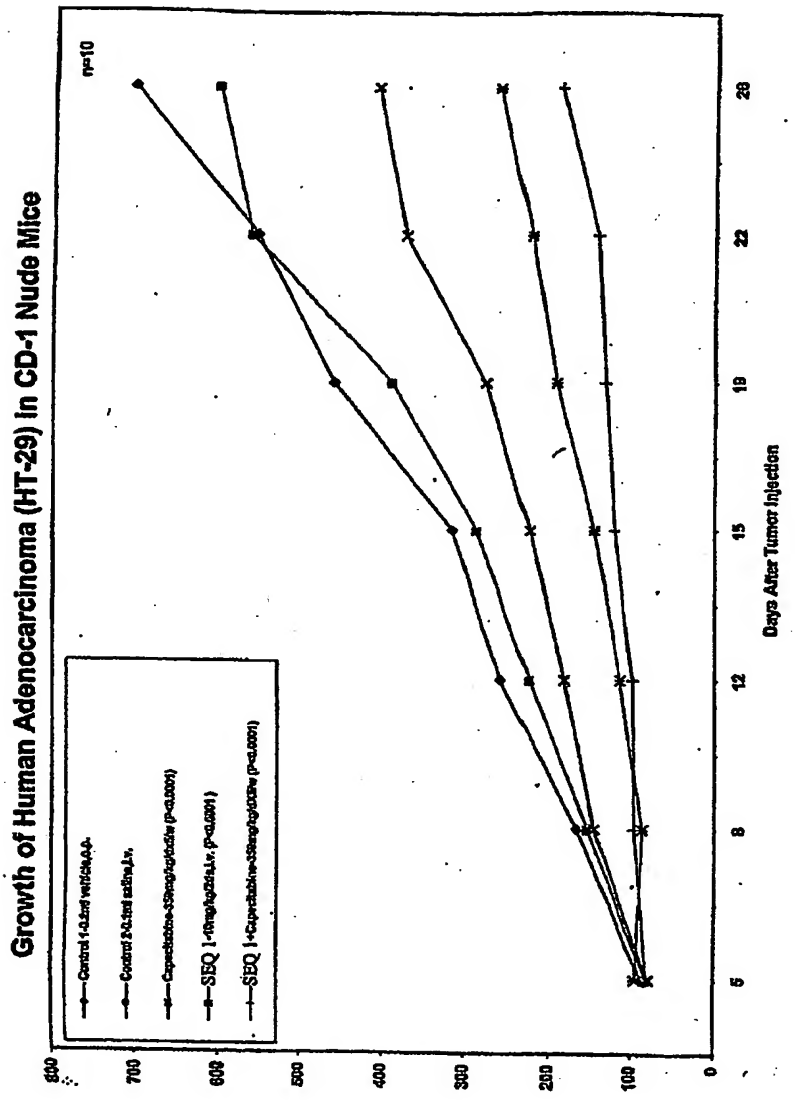


FIGURE 4

FIGURE 5

Pre-clinical Efficacy in Combination Therapy: Kidney Tumors (Caki-1)

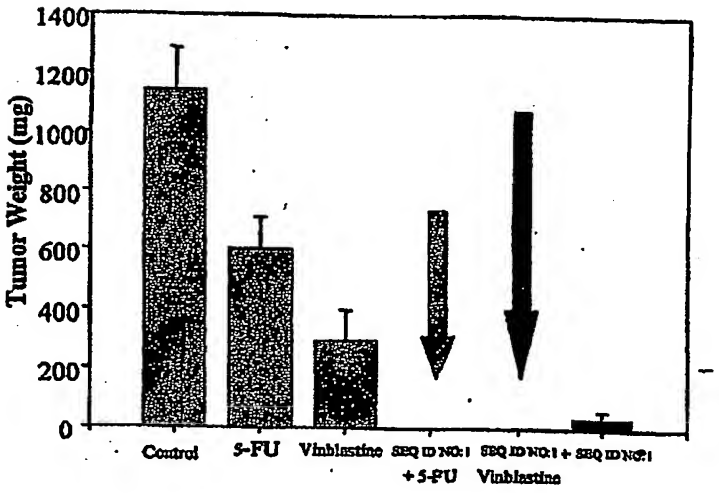


FIGURE 6

Weight of Human Prostate Carcinoma (PC-3) In SCID Mice

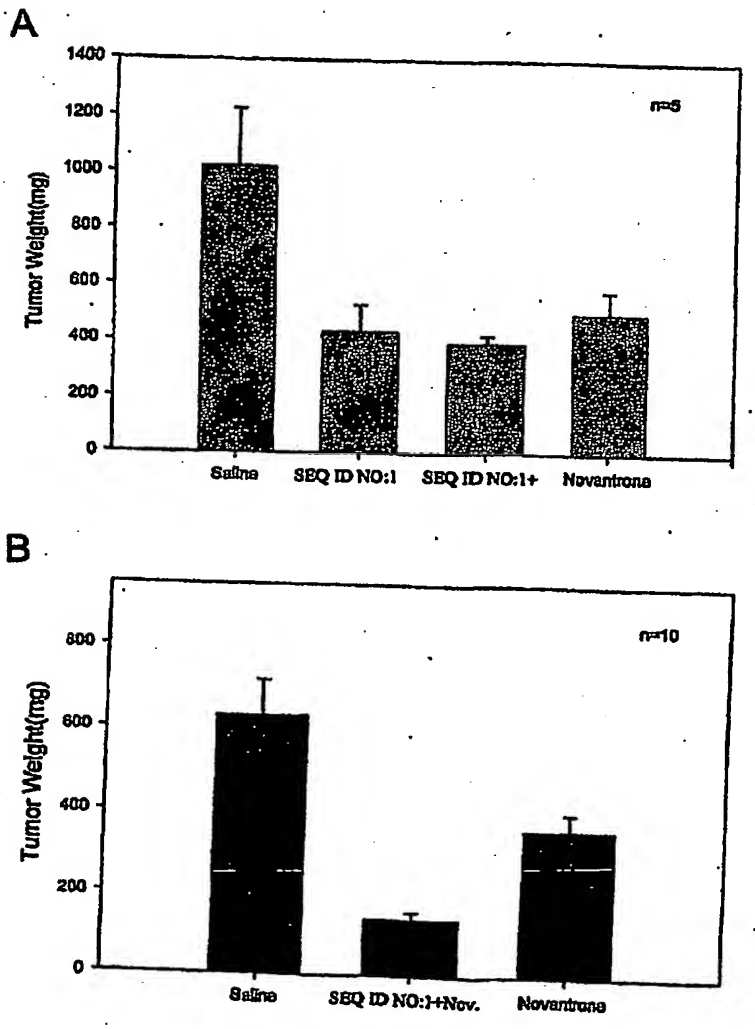


FIGURE 7

Weight of Human Prostate Carcinoma(DU145) in SCID Mice

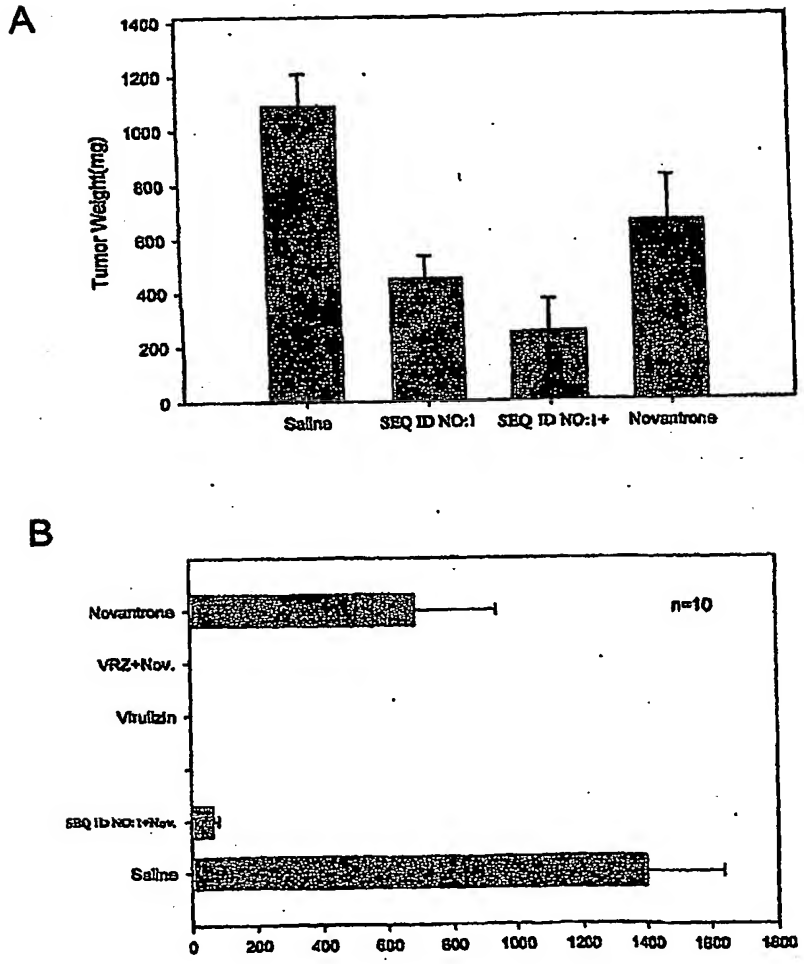


FIGURE 8

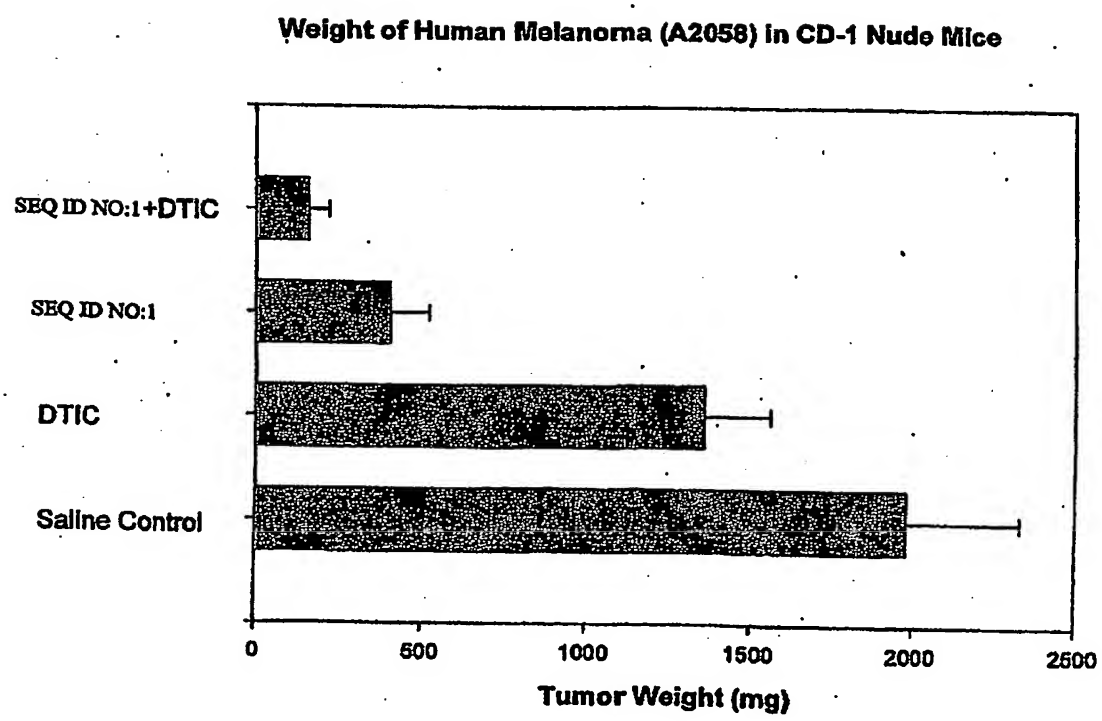
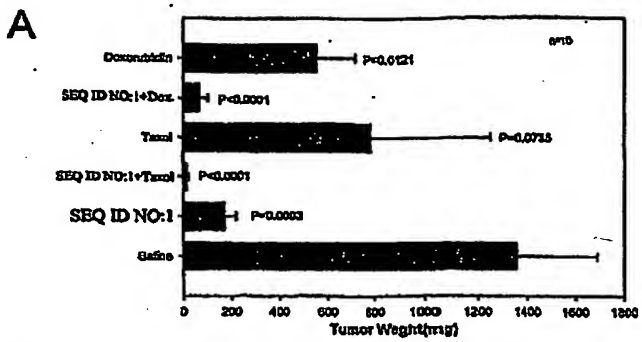
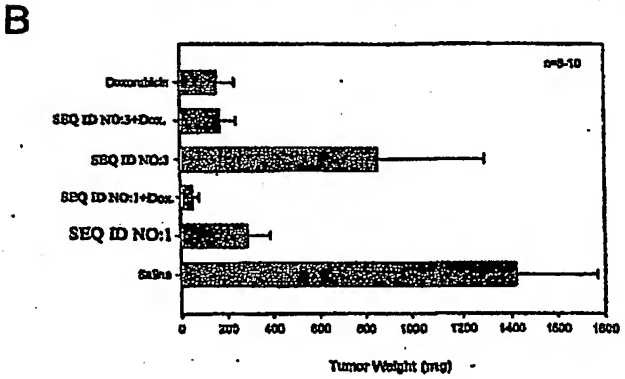


FIGURE 9

Weight of Human Breast Adenocarcinoma (MDA-MB-231) in CD-1 Nude Mice





Growth of Human Breast Adenocarcinoma (MDA-MB-231) in CD-1 Nude Mice

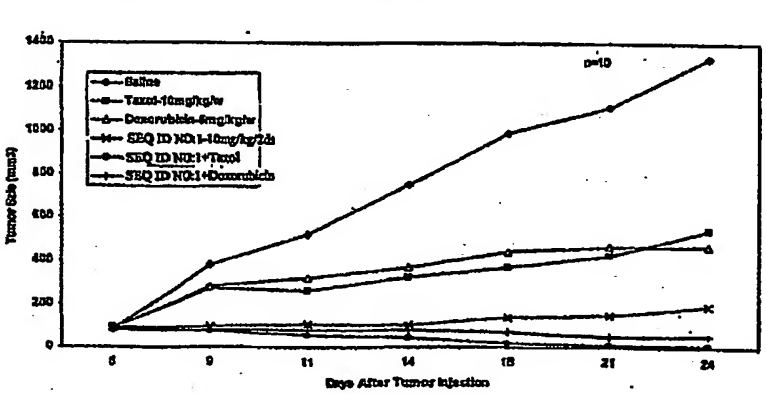


FIGURE 10

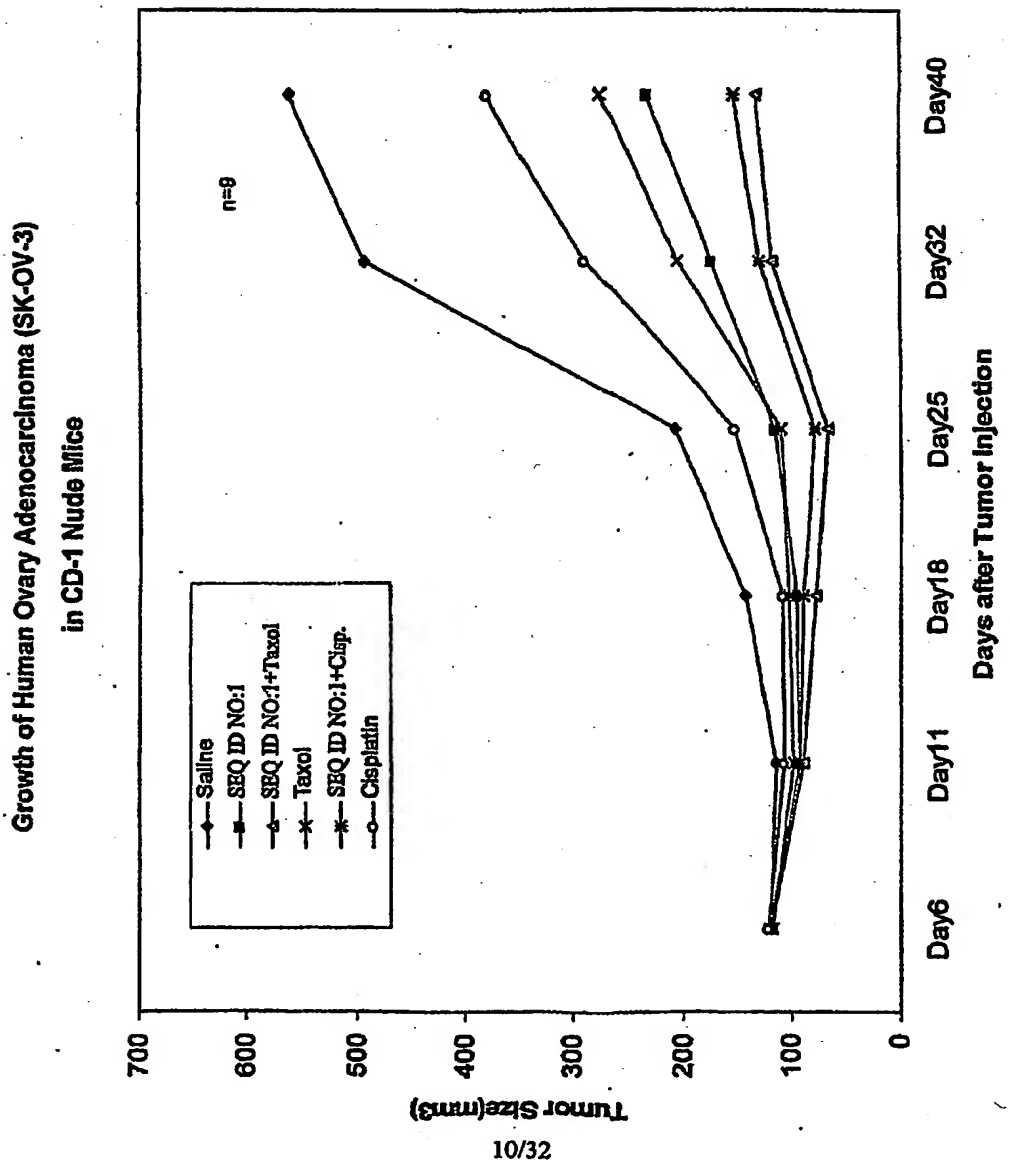
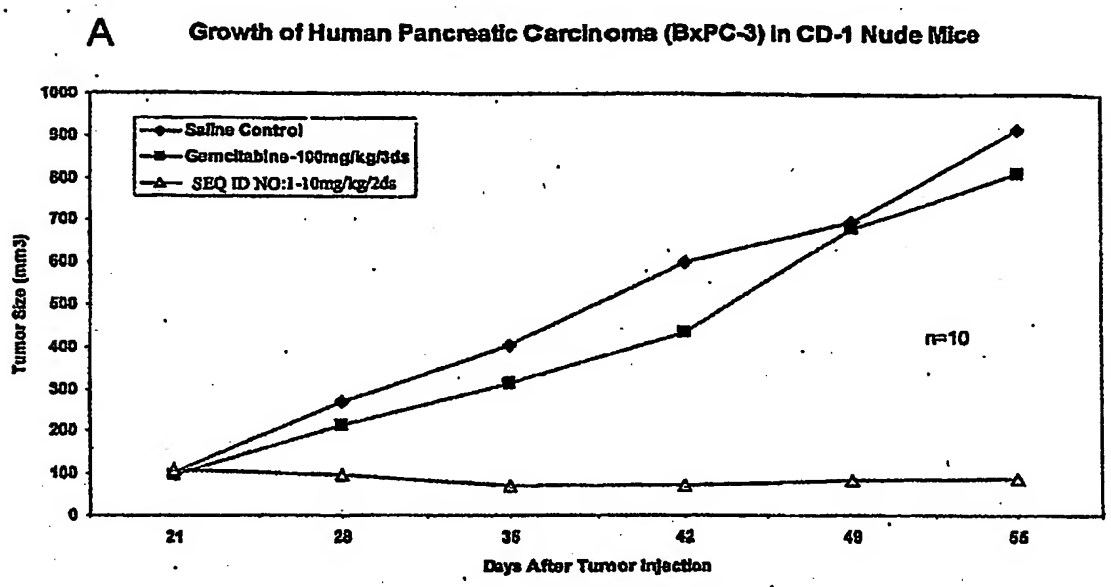


FIGURE 11



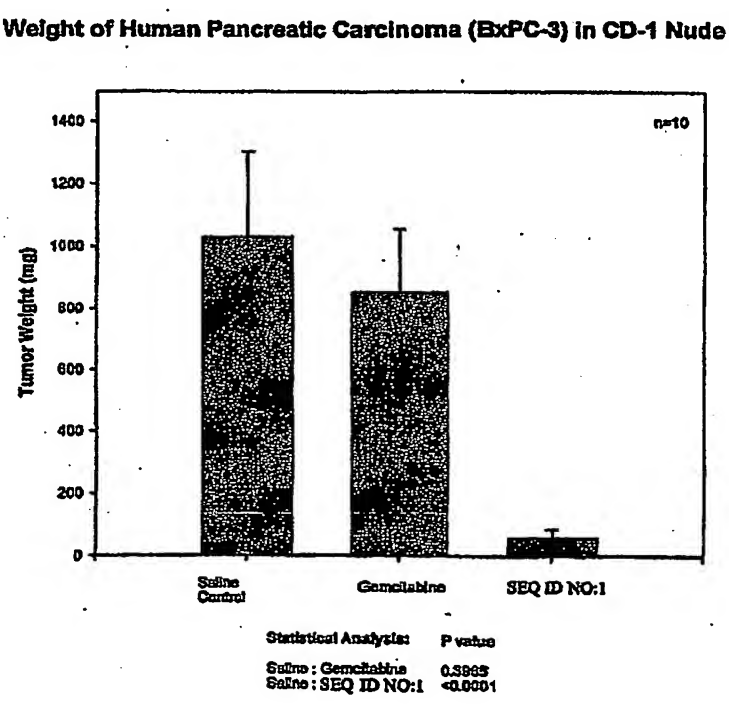
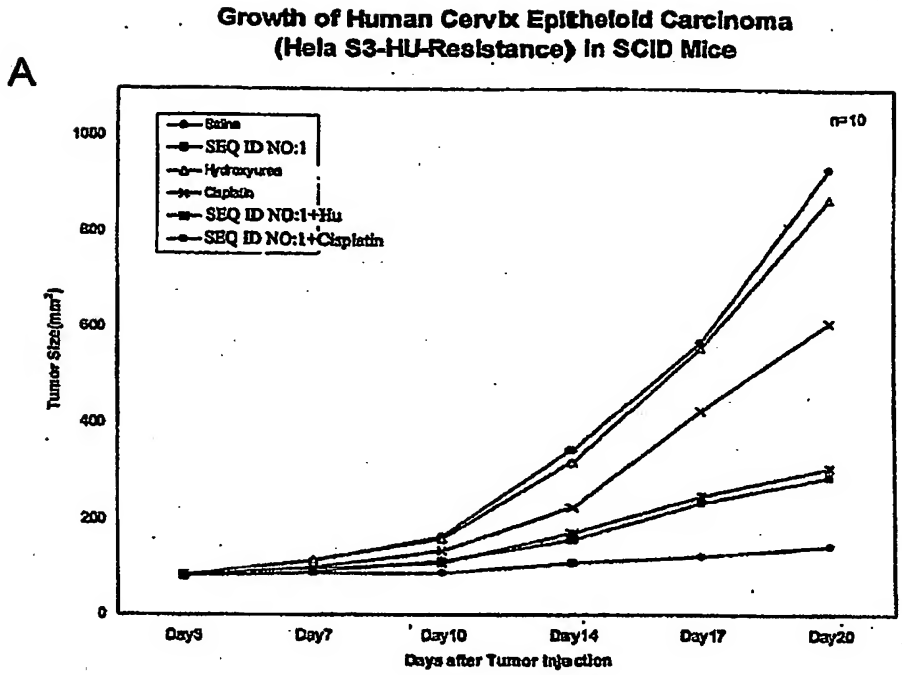


FIGURE 12



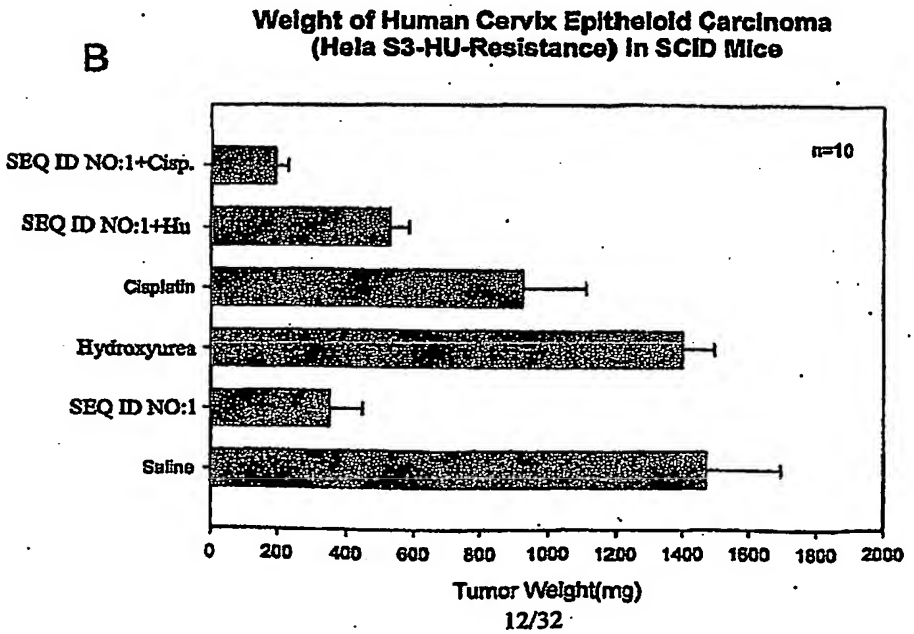
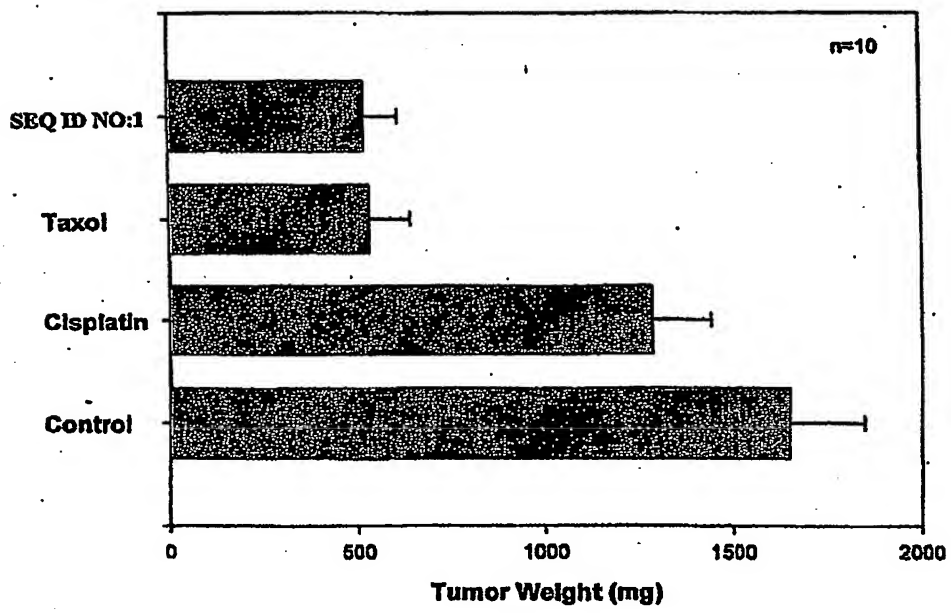


FIGURE 13

Weight of Human Cisplatin-Resistant Breast Adenocarcinoma Implanted at the Fat Pad of SCID Mice

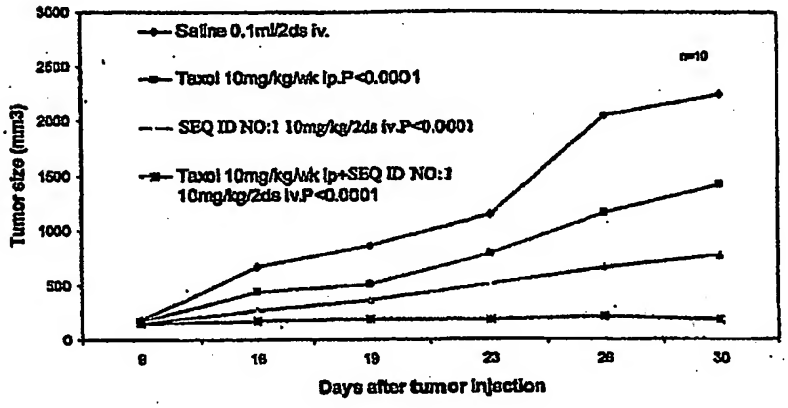


Statistical Analysis:	P value
Saline : Cisplatin	0.0834
Saline : Taxol	<0.0001
Saline : SEQ ID NO:1	<0.0001
SEQ ID NO:1 : Cisplatin	0.0007
SEQ ID NO:1 : Taxol	0.9547

FIGURE 14

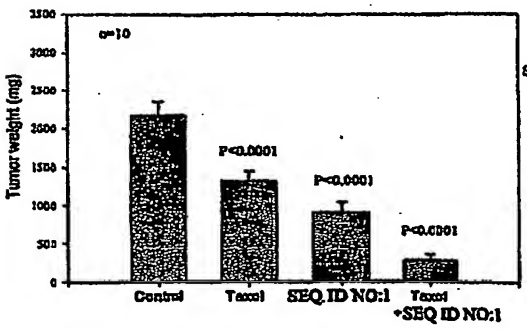
Growth Of Human Breast Cancer (MDA-CDDP-S4) In CB-17 SCID Mice Treated With Taxol, SEQ ID NO:1, and Taxol+ SEQ ID NO:1 (Orthotopical transplant)

A

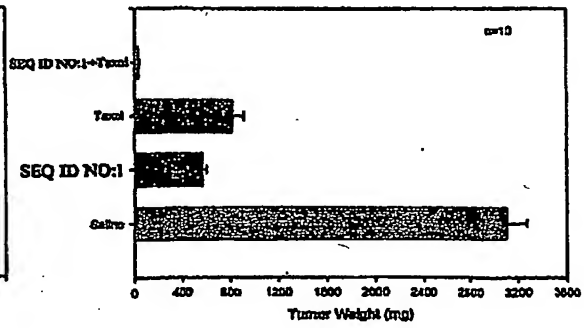


Tumor Weight Of Human Breast Cancer (MDA-CDDP-S4) In CB-17 SCID Mice Treated With Taxol, SEQ ID NO:1, and Taxol+SEQ ID NO:1

B



C



SEQ ID NO:1: Taxol P=0.0133
SEQ ID NO:1: SEQ ID NO:1+Taxol P=0.0003
Taxol : SEQ ID NO:1+Taxol P<0.0001

FIGURE 15

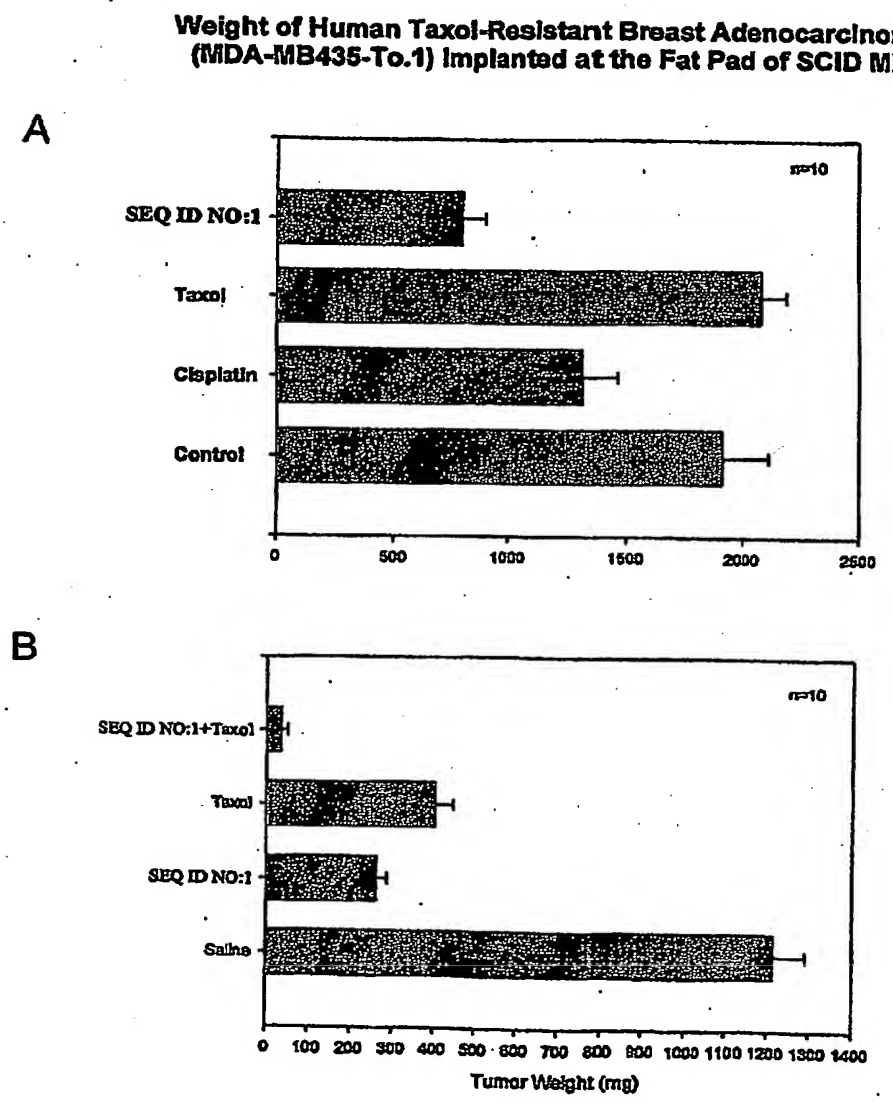
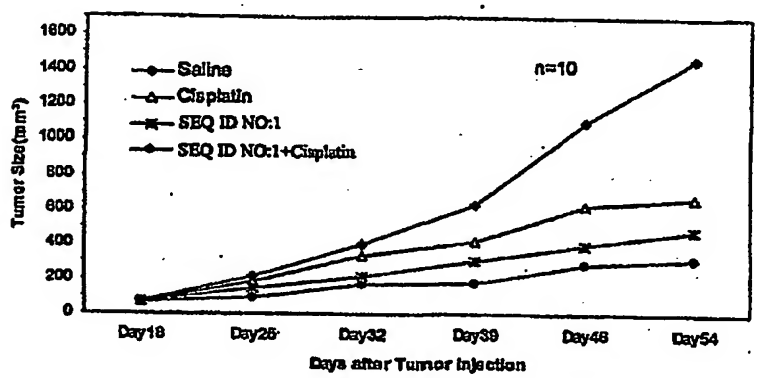


FIGURE 16

A



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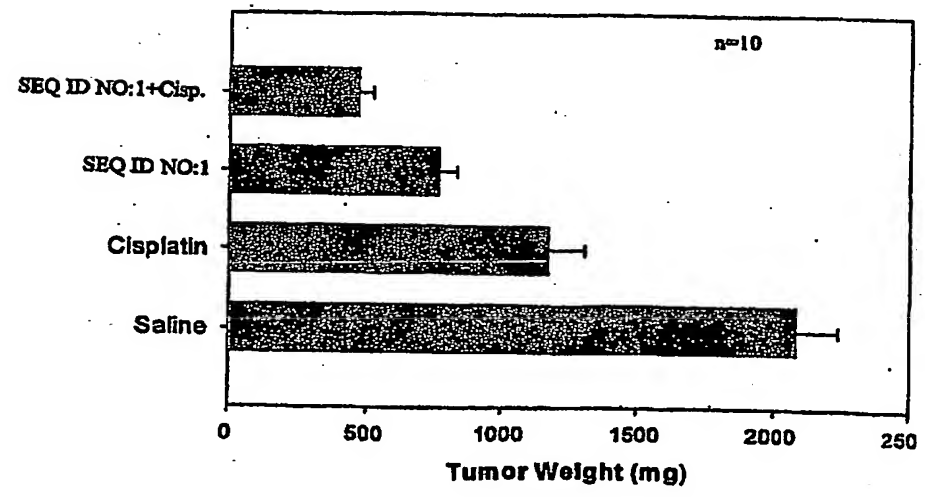
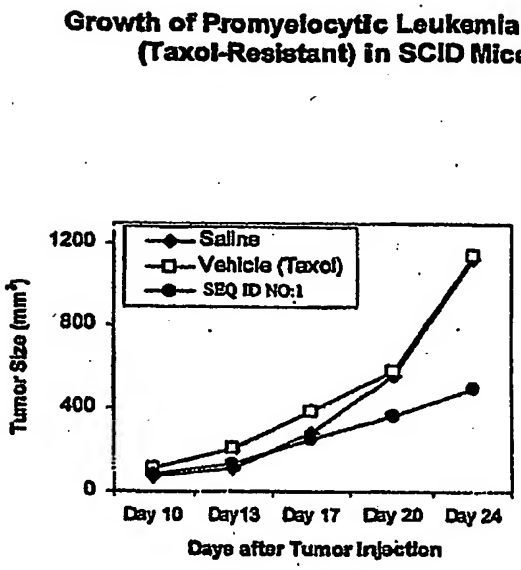


FIGURE 17

A



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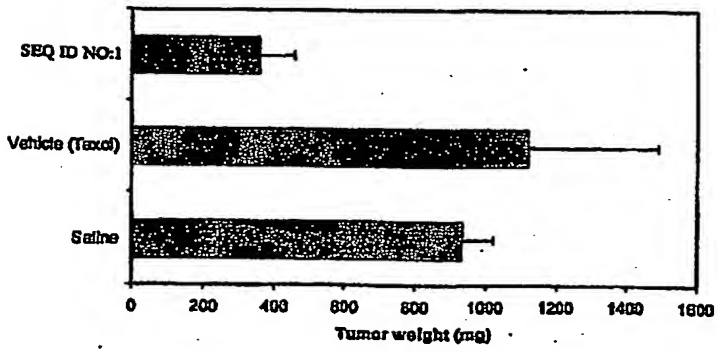
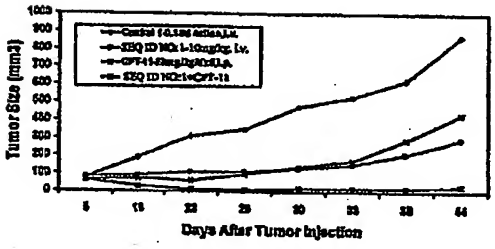


FIGURE 18

Growth of Human Multi-Drug Resistance Colon Adenocarcinoma (LS513) In SCID Mice

A



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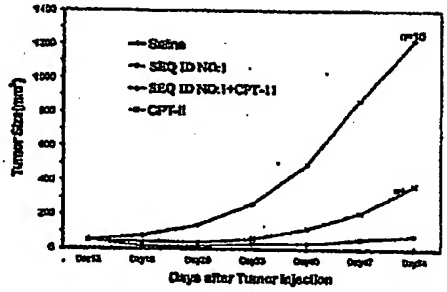
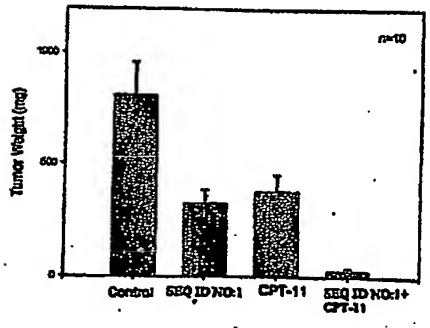


Weight of Human Colon Multi-Drug Resistance Carcinoma (LS513) In SCID Mice

C



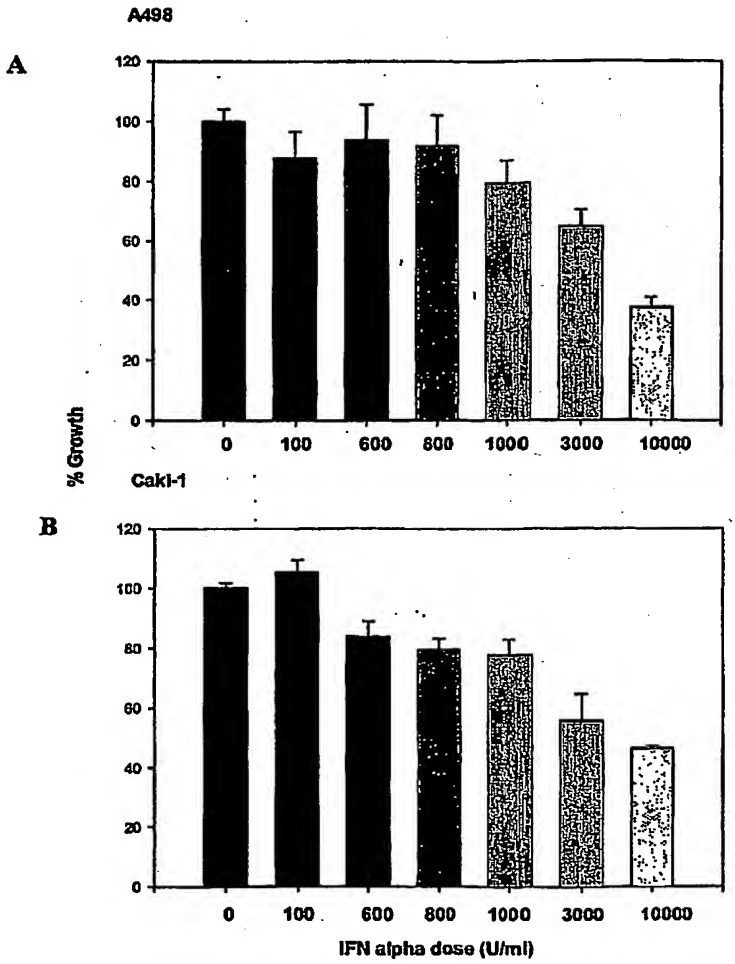


Figure 19

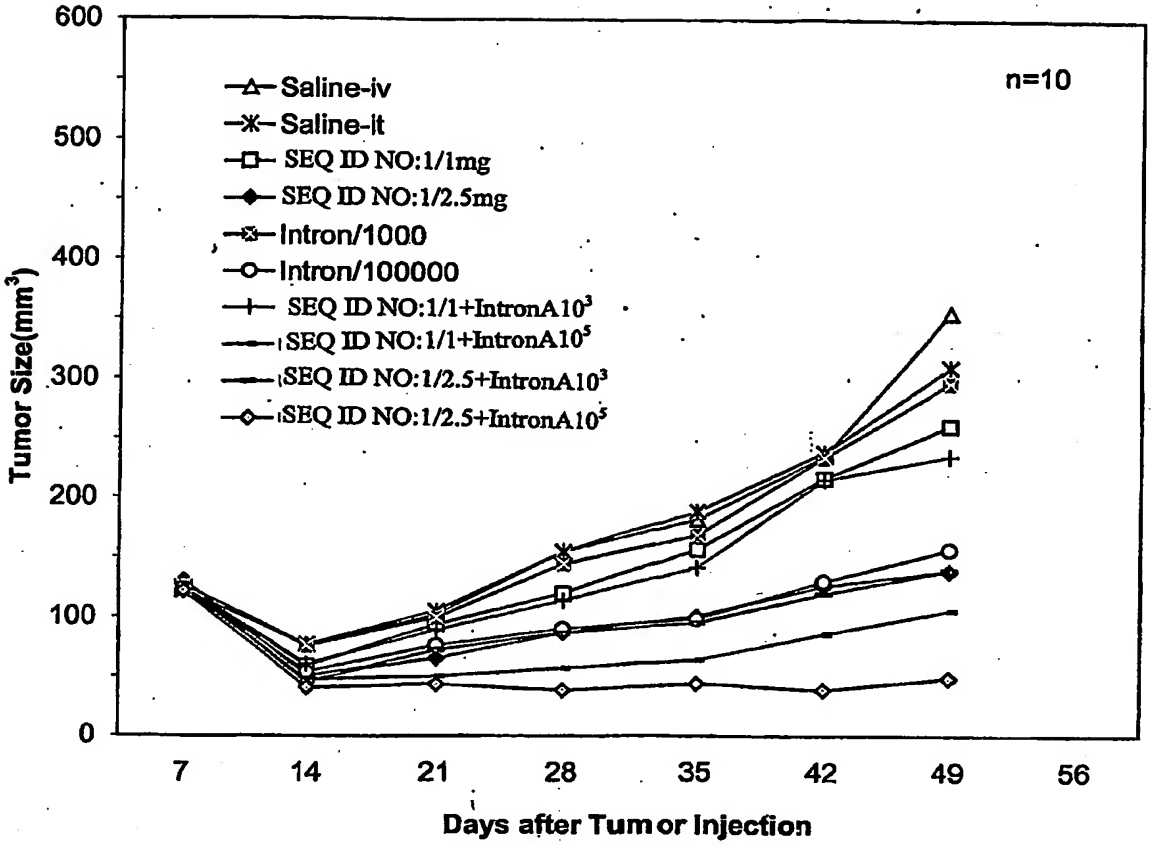
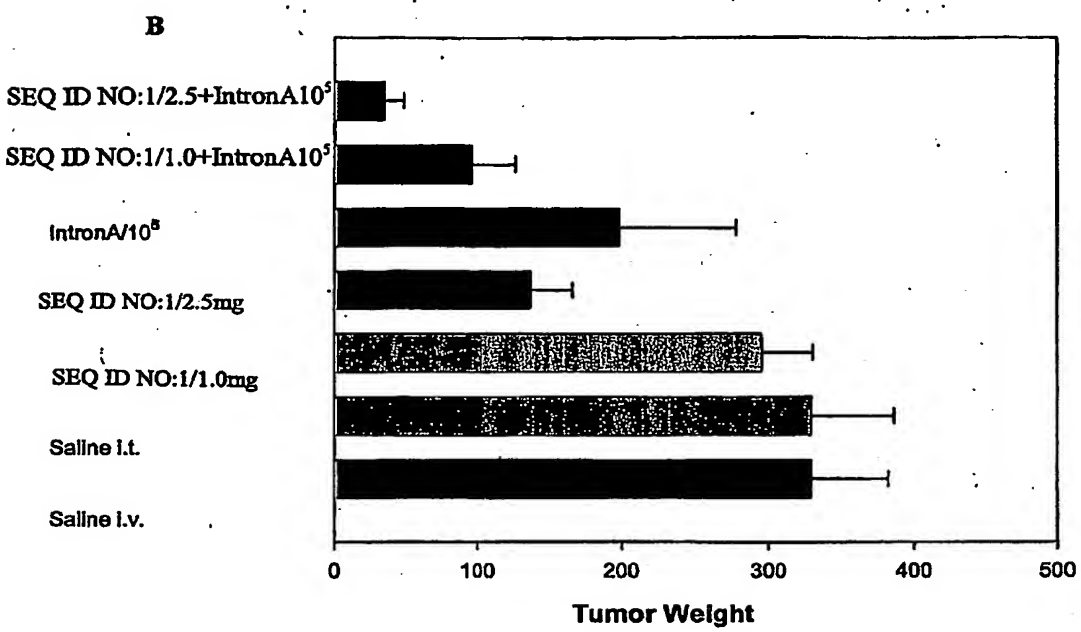


Figure 20 A



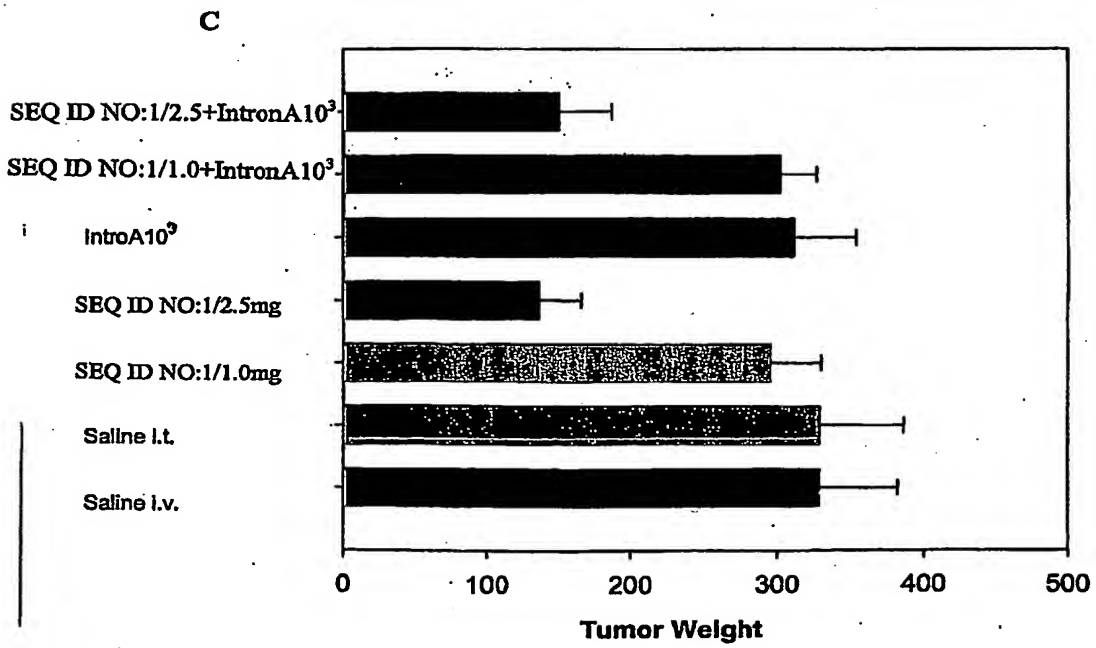


Figure 20 B & C

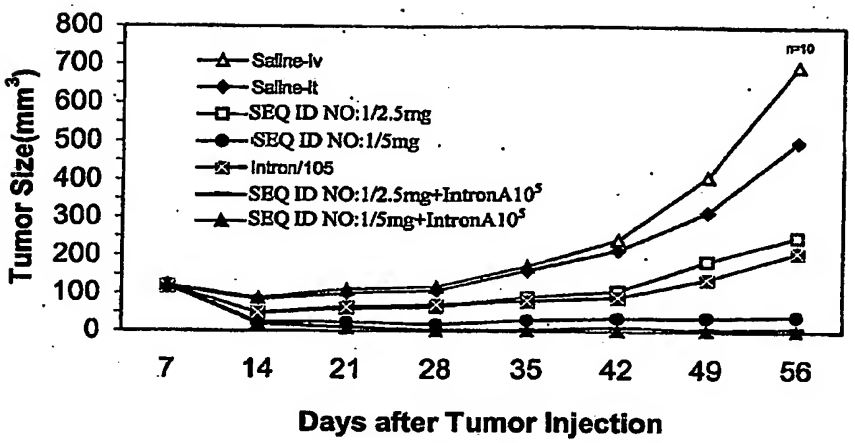


Figure 21 A

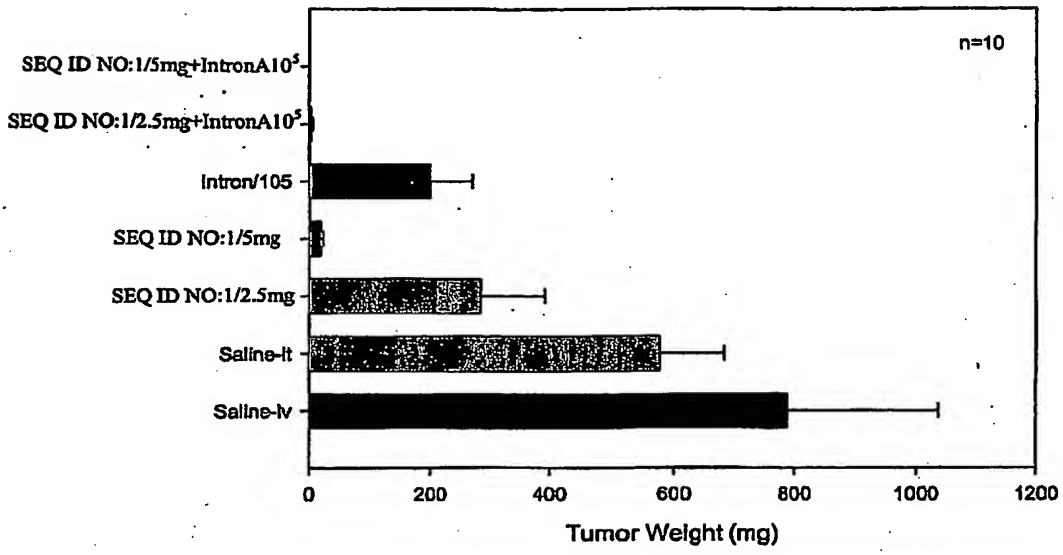


Figure 21 B

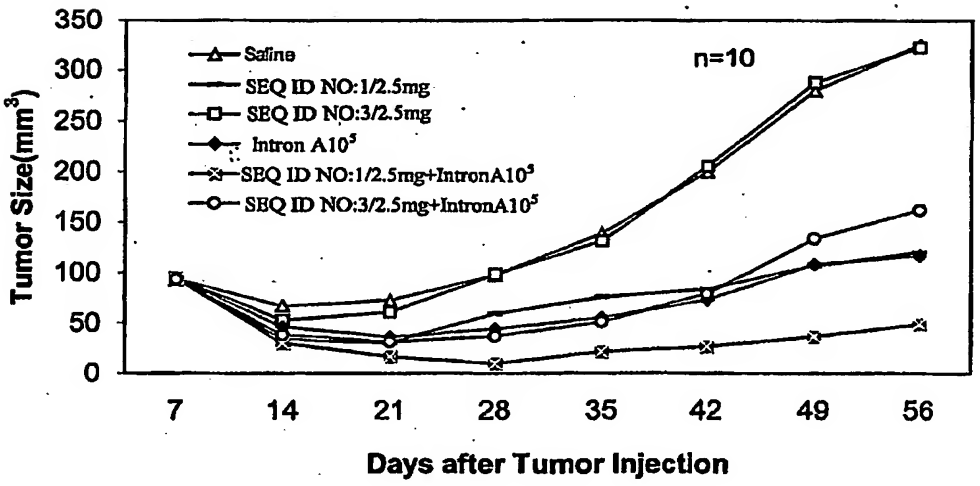


Figure 22A

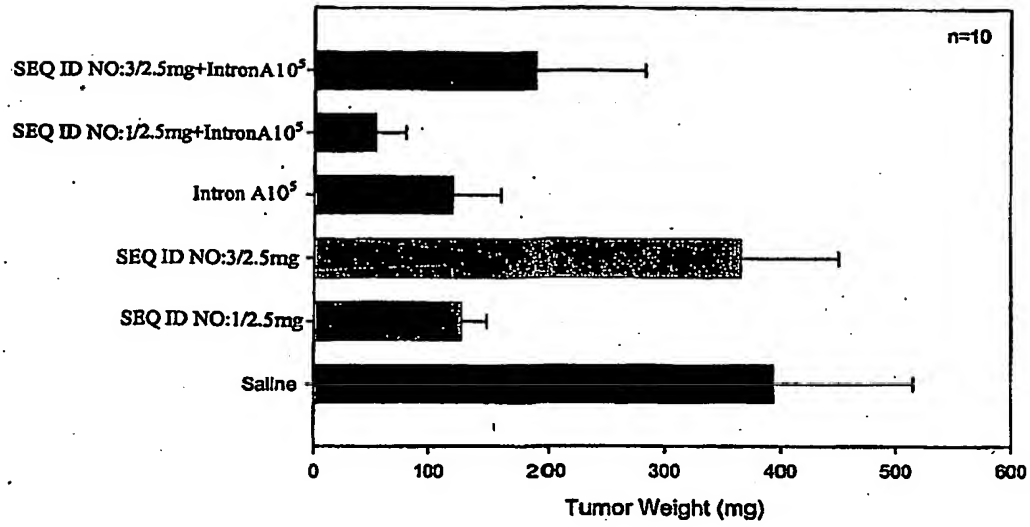


Figure 22B

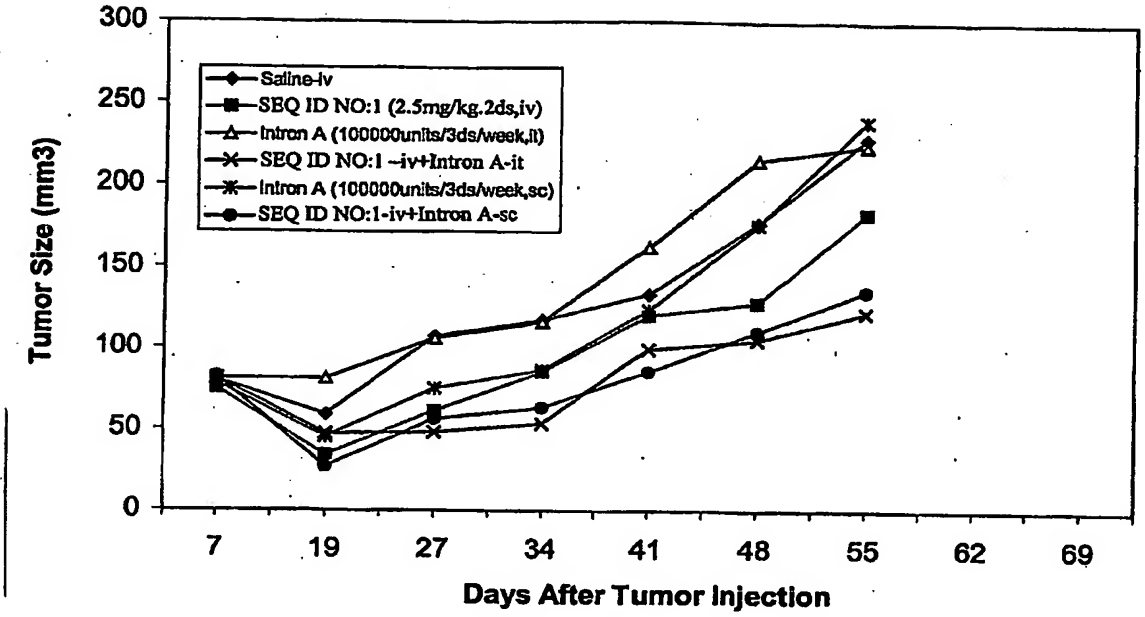


Figure 23

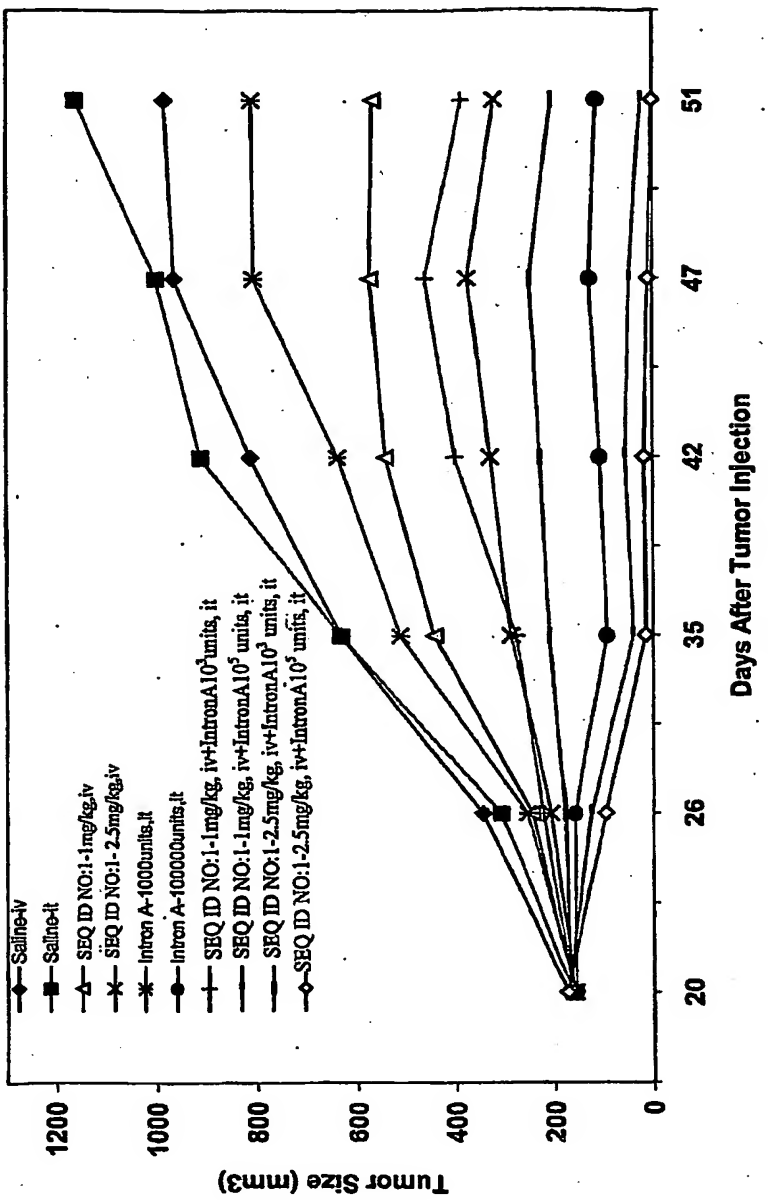
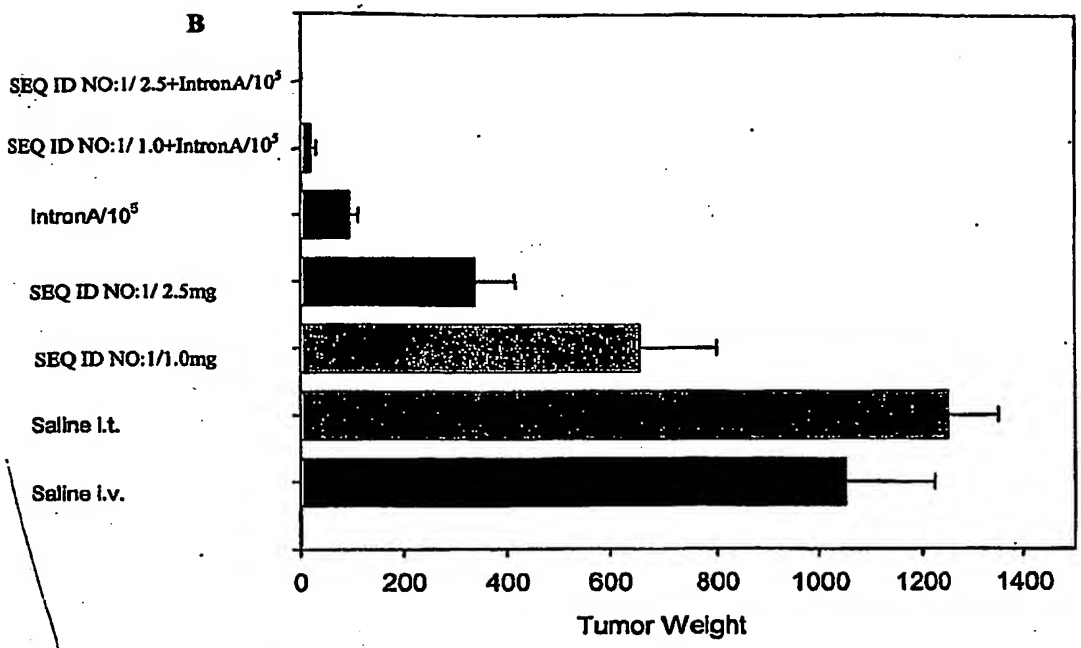


Figure 24A



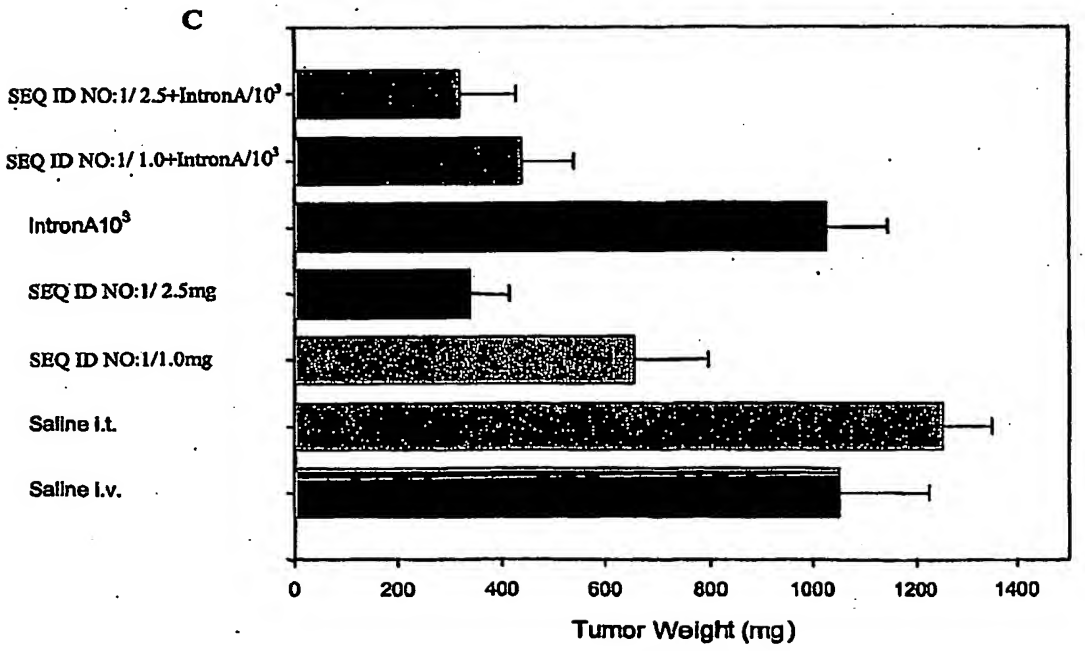


Figure 24

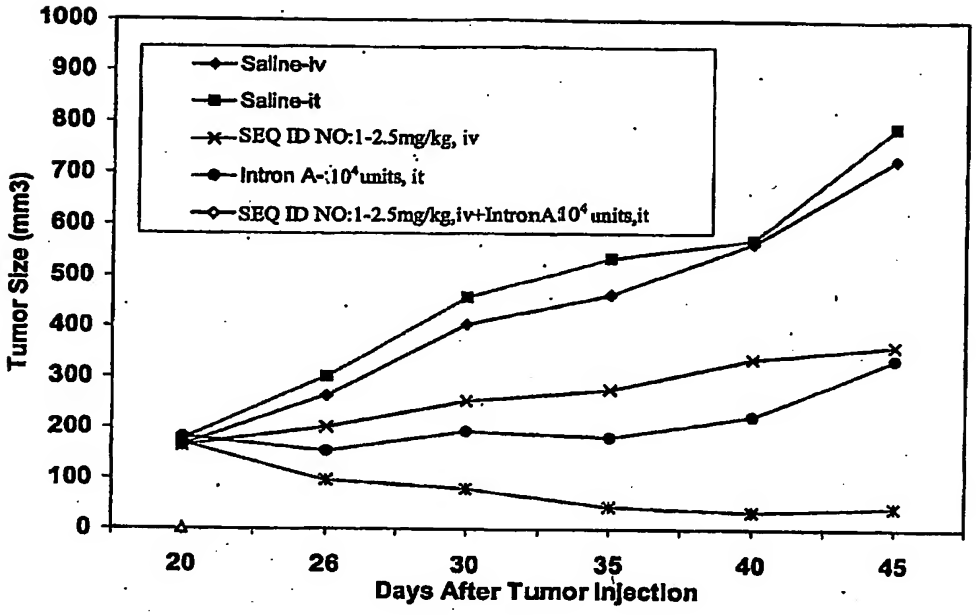


Figure 25A

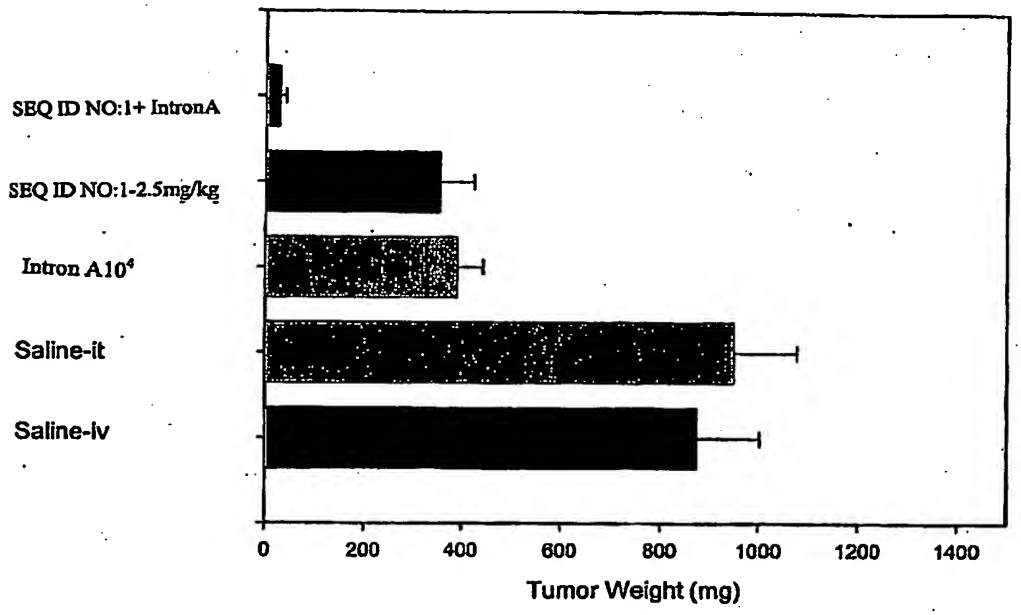


Figure 25B

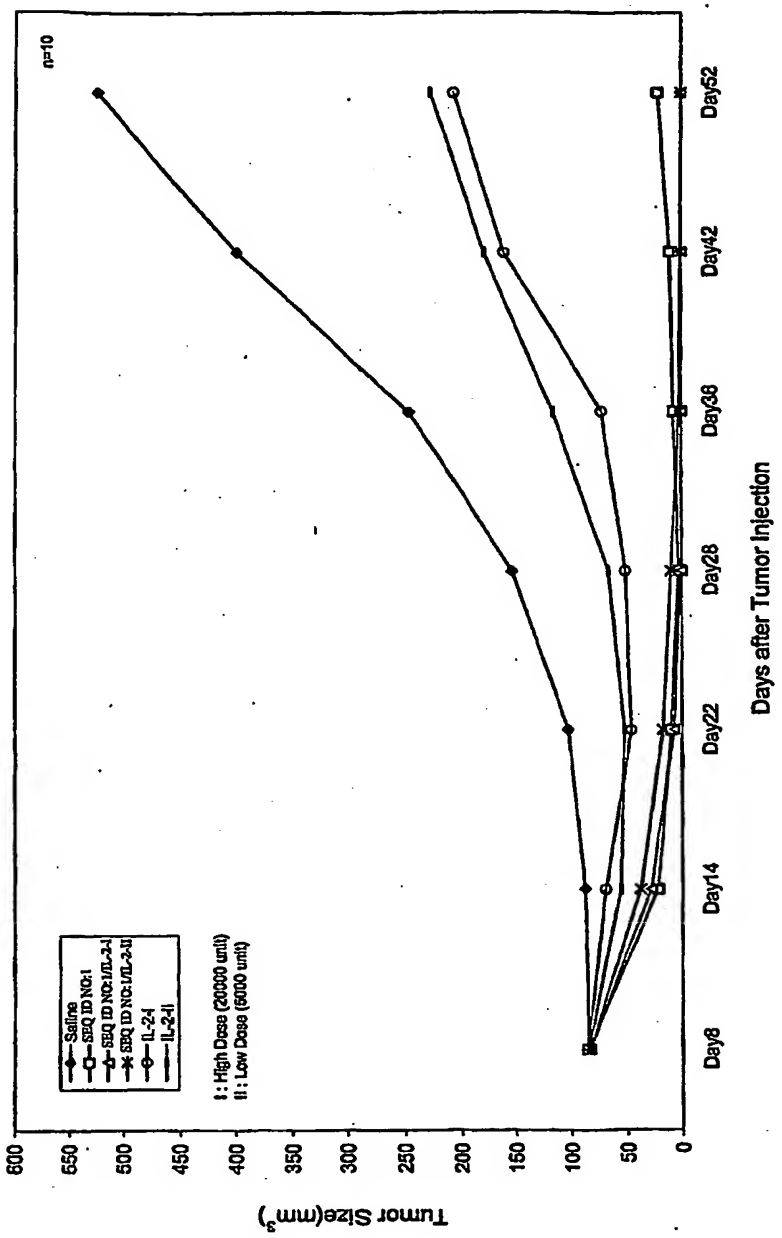


Figure 26A

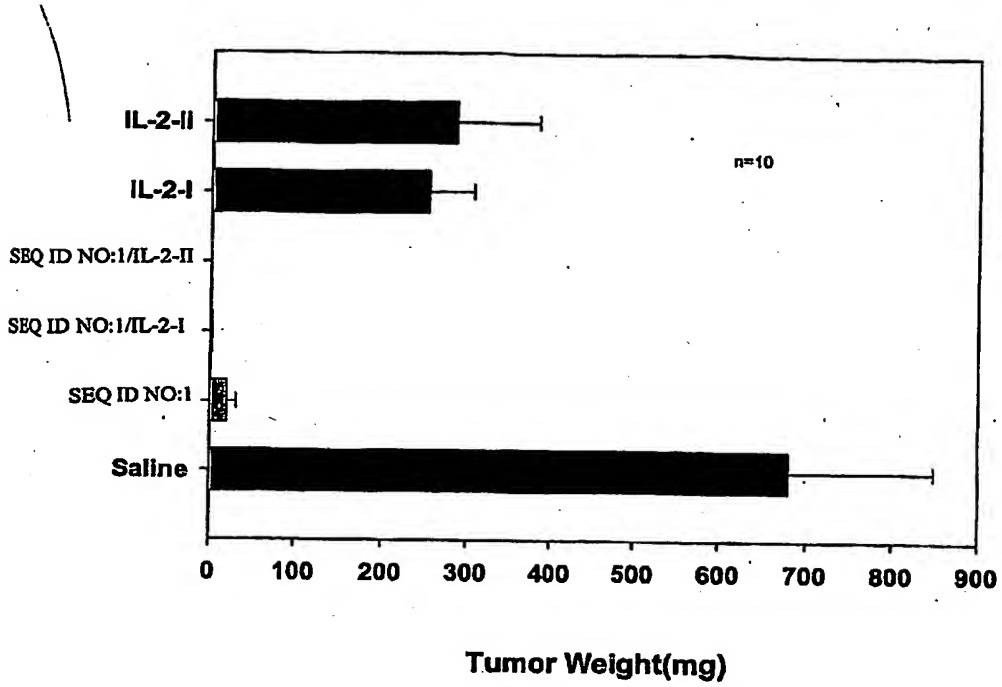


Figure 26B